Editor in Chief: IRVING S. WRIGHT, M.D.
Consulting Editor: RICHARD H. ORR, M.D.

The Megaloblastic Anemias

by VICTOR HERBERT, M.D.

Formerly Assistant in Hematology, The Mount Sinai Hospital, New York; Presently Research Associate, Thorndike Memorial Laboratory, Boston City Hospital, and Instructor in Medicine, Harvard Medical School



GRUNE & STRATTON • 1959
NEW YORK AND LONDON

MODERN MEDICAL MONOGRAPHS 18

Editor in Chief IRVING S WRIGHT M D
Consulting Editor RICHARD H ORR M D

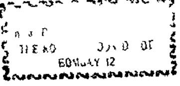
The Megaloblastic Anemias

By VICTOR HERBERT, M D

Research Assistant in Hematology The Mount Sinai Hospital New York Presently Research Associate Thorndike Memorial Laboratory Boston City Hospital and Instructor in Medicine Harvard Medical School



GRUNE & STRATTON • 1959
NEW YORK AND LONDON



The manuscript of this volume was awarded

HONORABLE MENTION

in the first Modern Medical Monographs Competition

381 Park Avenue South, New York 16, New York
Library of Congress catalog card number 59-10[illegible]62
Printed and bound in the United States of America (B)

Contents

Preface

APPROXIMATELY EIGHTY YEARS AGO Ehrlich[1] described a pathologic type of erythrocyte maturation in pernicious anemia This abnormal developmental line of erythrocytes was designated as the megaloblastic or large germ cell series to differentiate it from the normoblastic or normal sized germ cell series Anemia associated with this abnormal morphologic picture has become known as megaloblastic anemia

In the eight decades since Ehrlich s first description much has been learned about the nature and significance of megaloblastic blood cell formation The overwhelming majority of megaloblastic anemias have been found to be due to a deficiency of either vitamin B_1 or folic acid or both vitamins[3 4] When a megaloblastic morphologic picture has been observed in conditions other than frank deficiency of these vitamins the condition has been characterized by inhibition or exhaustion of the bone marrow with possible local deprivation of vitamin B_1 folic acid or building blocks for nucleoprotein synthesis in which vitamin B_1 and folic acid serve as co factors The accumulated information has made possible a unified concept of the megaloblastic anemias as a single morphologic entity due to defective nucleoprotein synthesis of various causes This monograph will be concerned with the development of megaloblastic anemia in morphologic and biochemical terms the causes of megaloblastic anemia as it occurs clinically application of this knowledge to accurate differential diagnosis of these anemias and a simple and rational approach to therapy based on the actual clinical problem as determined by proper differential diagnosis

This monograph is based in large measure on recent massive and productive research in the field of the megaloblastic anemias carried out and reported from all parts of the globe This is supplemented by the author s own research experiences and observations of more than 200 cases of megaloblastic anemia seen

at the Albert Einstein College of Medicine–Bronx Municipal Hospital Center the Montefiore Hospital and the Mount Sinai Hospital all in New York

The author is indebted to his mentors at each of these three eminent institutions Dr Irving M London Dr Theodore H Spaet and Dr Louis R Wasserman The freedom of inquiry and wise counsel they gave did much to make this monograph possible The author is also indebted to Dr William B Castle whose continued interest in the investigative work pursued by the author was a great sustaining force

Gratitude is also due the New York Heart Association and Heart Fund Inc and its Medical Director (now retired) Dr D F Milam for personal interest in and support of this work from its inception through four years of Research and Senior Research Fellowships given to the author Finally and by no means least United States Public Health Service Research Grants to Drs London Spaet Wasserman and the author as well as a grant from the National Vitamin Foundation and various other grants provided the equipment and supplies for much of the research related to the megaloblastic anemias with which the author was connected

The author would like to thank the staff of Grune and Stratton for their patience and editorial help

VICTOR HERBERT M D

1 *Incidence*

MEGALOBLASTIC ANEMIA is common all over the world, although the percentage of such anemias due to any specific cause varies widely from one geographic area to another and from one set of circumstances to another in any single area. As examples may be cited the prevalence of nutritional megaloblastic anemia in India,[5-8] Macedonia,[9] and Malaya,[10] the frequency of fish tapeworm megaloblastic anemia in Finland, especially widespread during World War II when particularly harsh conditions prevailed among the Finnish soldiers,[11] and the proneness of persons from the Scandinavian countries, England, Ireland, and Canada to develop classic addisonian pernicious anemia.[1]

The most common causes of megaloblastic anemia in the New York metropolitan area, where the author acquired most of his experience with the disorder, are addisonian pernicious anemia and malabsorption syndrome. The former is most common *in the majority of patients; the latter predominates among* the inhabitants of the city born in Puerto Rico. In all of the three New York hospitals with which the author has had major affiliation, approximately 10 per cent of the entire hematology department case load consisted of patients with megaloblastic anemia.* It is of interest in this connection that approximately the same percentage of the text of Wintrobe's *Clinical Hematology*[13] is devoted to the subject of the megaloblastic anemias, attesting to the importance of the subject to physicians in general and hematologists in particular.

The hematologic caseload of patients with megaloblastic anemia of the classic addisonian pernicious anemia variety shows every indication of a gradual future increase. This is a result of the fact that addisonian pernicious anemia is primarily a

* Of course, if the many cases of simple chronic anemia (as defined by Wintrobe) and iron deficiency anemia which are never referred to hematology departments were so referred, the percentage of hematology department patients with megaloblastic anemia might be less than 10 per cent of the total caseload.

disease of late adult life[14] and the average life expectancy of the population is increasing Addisonian pernicious anemia is very rare in children[15] rare in adults under 30 years and seldom seen prior to the age of 40[14]

It is presently believed that the decrease in serum vitamin B_{12} levels with aging is of dubious significance[16] However the increase in gastric atrophy with aging is not in doubt Gastric atrophy may be a factor in the decrease in serum vitamin B_{12} levels and in the eventual development in some cases of megaloblastic anemia due to vitamin B_{12} deficiency[17, 18] (See section Hereditarily Determined Degenerative Gastric Atrophy page 21)

2 *Hematologic Morphology*

IN A WELL DEVELOPED CASE of megaloblastic anemia the picture presented by both the peripheral blood and the bone marrow is usually so characteristic as to be impossible to confuse with the morphologic picture of any other disorder. In the peripheral blood are found many large and frequently oval erythrocytes (macrocytes and macroovalocytes, the latter often referred to as "megalocytes" by European clinicians and morphologists). The nuclei of the neutrophilic polymorphonuclear leukocytes are hypersegmented,[1, 2] as often are the nuclei of the eosinophils; oval neutrophils may be seen; and in addition to frequently being obviously reduced in number, many of the platelets may be giant and bizarre in appearance.[3]

This picture is accompanied by a decrease in the total number of erythrocytes and neutrophilic polymorphonuclear leukocytes and an increase in the mean corpuscular volume (MCV) of the erythrocytes due to the increased size of the average red blood cell. FIGURE 1 shows the typical appearance of the peripheral blood of a patient with megaloblastic anemia. With respect to the red blood cell morphology, it should be noted that, aside from the characteristic macrocytosis and macroovalocytosis, marked anisocytosis and poikilocytosis are frequently not present when the anemia is not severe.

The reticulocytes in the peripheral blood usually represent about 1 per cent of the total number of circulating erythrocytes. This is probably a consequence of the fact that the asynchronism in development of nucleus and cytoplasm of the erythrocyte precursors in the bone marrow results in frequent disappearance of cytoplasmic reticulum even before the nucleus is extruded. Thus more than half of the erythrocytes delivered to the peripheral blood from the bone marrow may be without reticulum. In megaloblastic anemia, therefore, the reticulocyte count provides an erroneously low figure of the rate of red cell production.[4]



Fig 1 — The peripheral blood in megaloblastic anemia (From DALAND C A HA L T H AND FIOTTE E A Color Atlas of Morphologic Hematology Cambridge Harvard University Press 19 1)



Fig —M gal bla t devel pment of the er thr c t

The bone marrow displays megaloblastic development of the erythrocytes as shown in FIGURE 2 By comparison with normal (normoblastic) erythrocyte maturation the megaloblastic series presents a larger size at every stage during development with a larger amount of cytoplasm in proportion to the size of the nucleus The nucleus itself is very large compared to the nucleus of a normoblast and there is a striking disparity between the apparent maturity of the nucleus and that of the cytoplasm This disparity or nuclear cytoplasmic dissociation or asynchronism as it is variously termed [5-7] is reflected in the much more particulate nuclear chromatin (young nucleus or nucleus with retarded maturation) [8] of the megaloblasts at all stages of their development even when hemoglobin is clearly visible in the cytoplasm (old cytoplasm)[2, 9] The megaloblasts with their finely particulate young chromatin are easily distinguished from normoblasts with their coarsely clumped old nuclear chromatin especially in those cells where hemoglobinization of the cytoplasm is visibly beginning

Another striking feature of the bone marrow morphology in megaloblastic anemia is a megaloblastic polymorphonuclear leukopoiesis manifested most dramatically by the presence of many giant metamyelocytes as depicted in FIGURE 3

There is usually a marked increase in the number of mitotic and binucleate cells in the erythroid series

The degree of overt megaloblastosis of the bone marrow appears generally to be proportional to the severity of the anemia Study of patients after total gastrectomy [0] demonstrated the evolutionary sequence in the development of megaloblastic anemia to be macrocytosis first anemia second (after an average delay of one or two years following the appearance of macrocytosis) and megaloblastic marrow third and last

Note that the first hematologic evidence of vitamin B_{12} deficiency is macrocytosis (and an Arneth count shift to the right) with the bone marrow presumably remaining normoblastic until two or three years later Of course the macrocytes must have come from abnormal precursors and in every case of



Fig 1 — The peripheral blood in megaloblastic anemia (From Daland G A Ham T H and [illegible] F A Color Atlas of Morphologic Hematology Cambridge Harvard University Press 19[illegible]1)



Fig — Megaloblast [illegible]

The bone marrow displays megaloblastic development of the erythrocytes as shown in FIGURE 2 By comparison with normal (normoblastic) erythrocyte maturation the megaloblastic series presents a larger size at every stage during development with a larger amount of cytoplasm in proportion to the size of the nucleus The nucleus itself is very large compared to the nucleus of a *normoblast and there is a striking disparity between the* apparent maturity of the nucleus and that of the cytoplasm This disparity or nuclear cytoplasmic dissociation or asynchronism as it is variously termed [5-7] is reflected in the much more particulate nuclear chromatin (young nucleus or nucleus with retarded maturation) [8] of the megaloblasts at all stages of their development even when hemoglobin is clearly visible in the cytoplasm (old cytoplasm) [9] The megaloblasts with their finely particulate young chromatin are easily distinguished from normoblasts with their coarsely clumped old nuclear chromatin especially in those cells where hemoglobinization of the cytoplasm is visibly beginning

Another striking feature of the bone marrow morphology in *megaloblastic anemia is a megaloblastic polymorphonuclear* leukopoiesis manifested most dramatically by the presence of *many giant metamyelocytes as depicted in FIGURE 3*

There is usually a marked increase in the number of mitotic and binucleate cells in the erythroid series

The degree of overt megaloblastosis of the bone marrow appears generally to be proportional to the severity of the anemia Study of patients after total gastrectomy [30] demonstrated the evolutionary sequence in the development of megaloblastic anemia to be macrocytosis first anemia second (after an average delay of one or two years following the appearance of macrocytosis) and megaloblastic marrow third and last

Note that the first hematologic evidence of vitamin B_{12} deficiency *is macrocytosis (and an Arneth count shift to the right)* with the bone marrow presumably remaining normoblastic until two or three years later Of course the macrocytes must have come from abnormal precursors and in every case of

early vitamin B_{12} deficiency disease which the author has seen careful search of the bone marrow smear has revealed the presence of intermediate megaloblasts and large metamyelocytes. It is *probable* that these marrow findings are always present when the peripheral blood contains macroovalocytes and neutrophilic



FIG 3.—Giant metamyelocyte in a megaloblastic bone marrow. (Note also the pinching of the nuclei of the smaller and more mature metamyelocytes into multiple segments resulting in the typical hypersegmented polymorphonuclear leukocytes associated with megaloblastic anemia.)

polymorphonuclear leukocytes whose nuclei show an increased amount of segmentation even though most workers do not comment in print about abnormalities in the bone marrow until it is frankly megaloblastic. More will be said about the intermediate megaloblast shortly. For the present suffice it to say that emphasis on the macroovalocyte and the hypersegmented polymorphonuclear leukocyte as the first indicators of a developing megaloblastic anemia is quite proper since they are usually easily recognized and are found in the peripheral blood. Recognition of intermediate megaloblasts and large metamyelocytes

however requires aspiration of the bone marrow and a fair amount of experience in looking at bone marrow morphology. It is wise for the person who does not examine blood and bone marrow preparations daily to compare both the peripheral blood and the bone marrow of the patient with that of a normal control subject. If he does not do this he will miss many cases of early megaloblastic anemia since he will not recognize the early hematologic changes.[13]

Darby et al.[31] believe that macrocytosis is the most sensitive indicator of vitamin B_{12} deficiency. Their long-term observations of patients with pernicious anemia receiving smaller than minimal maintenance dosages of vitamin B_{12} indicated that the quantity of the vitamin needed for hematopoiesis exceeded that needed for other clinically recognizable physiologic functions. In the author's experience an Arneth count shift to the right invariably accompanies the macrocytosis and may even precede it.

A single case was reported suggesting that vitamin B_{12} deficiency may be manifested by an increase in the number of giant nuclei in the oral epithelial cells even before macrocytic erythrocytes make their appearance. The patient had overt neurologic damage, a serum vitamin B_{12} level of 60 $\mu\mu g$/ml (L. leishmanii assay), a serum iron concentration of 140 μg/100 ml, and definitely was not receiving folic acid therapy. It was stated that there was "no hypersegmentation of granulocytes," but an Arneth count was not reported. A photograph of the patient's peripheral blood smear was presented and interpreted by the authors as normal except for slight anisocytosis, but it appeared suggestive of macrocytosis and at least one of the erythrocyte looked like a macroovalocyte (megalocyte). The hematocrit and red cell count were not obtained, so the MCV could not be calculated.

In the absence of folic acid administration the author has never seen a patient having a serum vitamin B_{12} level below 100 $\mu\mu g$/ml without incipient or overt iron deficiency who did not have at least macroovalocytosis and an Arneth count shift

to the right in the peripheral blood and at least intermediate megaloblasts and large metamyelocytes in the bone marrow

Dr Boen generously made the patient's original peripheral blood and bone marrow smears available for examination The peripheral blood showed only one or two macroovalocytes per oil immersion field but an Arneth count revealed a definite shift to the right with 14 per cent of the neutrophilic polymorphonuclear leukocytes having more than 5 lobes The bone marrow contained a large number of large metamyelocytes and an occasional intermediate megaloblast but no megaloblasts A generalized pallor of the erythrocytes suggested a possible concomitant iron deficiency despite the normal serum iron level (Such a deficiency would explain the lack of a striking macroovalocytosis in the peripheral blood)

Thus this patient did have hematologic changes as well as epithelial cell changes and the case illustrated once again the importance of the Arneth count in helping to ascertain the existence of vitamin B_1 and or folic acid deficiency The question as to whether macrocytic changes due to deficiency of vitamin B_1 may appear first in the oral epithelial cells or the hematic cells remains open Theoretically one would expect macrocytic changes to appear first in those cells which normally proliferate most rapidly Concomitant iron deficiency might mask the macrocytic changes in the erythrocytes without masking the changes in the oral epithelial cells whose iron requirement is much lower

Recognition of the earliest hematologic signs of incipient megaloblastic anemia is particularly important because severe nervous system damage may occur in vitamin B_1 deficient patients prior to development of a megaloblastic marrow and even before anemia appears[31] The earlier such cases are recognized and proper treatment instituted the less irreversible damage will be done (See CHAPTER 6 Clinical Picture page 66 for cases in point)

The process of development of megaloblastic anemia may take years probably because the body is able to draw on its

liver stores of vitamin B_1 [34, 38] and folinic acid [34, 39] when the exogenous supply is cut off A simple screening procedure generally applicable to all patients on a routine basis and making it possible to recognize incipient megaloblastic anemia would be of great value Such a procedure has long been at hand and has been applied with marked success at the Montefiore Hospital where in the course of a single year it increased the number of newly discovered cases of vitamin B_1 deficiency disease from one every six weeks to one every six days [40] In fully one third of these cases no anemia was present and the diagnosis was not even remotely suspected prior to the routine screening the patients had been admitted for evaluation of unrelated conditions The positive result of the screening procedure led to confirmation of the diagnosis of early vitamin B_1 deficiency by determination of the serum vitamin B_1 level and Schilling type urinary excretion testing

This screening procedure consists simply in obtaining a routine peripheral blood smear on every new patient and carefully examining that smear for macroovalocytes and hypersegmented polymorphonuclear leukocytes All of the technicians in the routine clinical hematology laboratory are instructed that the normal average number of lobes of the nuclei of neutrophilic polymorphonuclear leukocytes is 3 that about 40 to 50 per cent normally have 3 lobes 20 to 40 per cent have 2 lobes and 15 to 25 per cent have 4 lobes [41] The finding of more than 3 5 lobed polymorphonuclears per hundred or even a single polymorphonuclear with more than 5 lobes or a substantial increase in the percentage with 4 lobes signals a careful work up for incipient megaloblastic anemia even when absolutely no anemia is demonstrable An increase in the number of polymorphonuclear leukocytes with 4 or more lobes or Arneth count shift to the right [illegible] has preceded the onset of anemia in every case of incipient megaloblastic anemia this author has observed Others [13] also have observed and reported on this phenomenon

Frequently in incipient megaloblastic anemia many of the hypersegmented polymorphonuclears look at first glance like

normal young polymorphonuclears with horseshoe or sausage shaped nuclei. More careful inspection reveals that what appears to be a single segment is in fact a rosette of overlapping segments or rather a sausage which is being pinched into a number of small *round sausages*. These abnormal young polymorphonuclears when in the bone marrow are the immediate precursors of the typical hypersegmented polymorphonuclear leukocytes usually seen in the peripheral blood[4] (See FIGURE 3.)

When macroovalocytes and hypersegmented polymorphonuclear leukocytes are found in the peripheral blood, examination of the bone marrow obtained by aspiration biopsy should be the next step. Even if a megaloblastic marrow is not found in such circumstances, however, work up of the patient for an incipient megaloblastic anemia will be fruitful in the large majority of cases. It cannot be emphasized too strongly that in cases of incipient megaloblastic anemia, even highly trained hematologists may not find clearly megaloblastic primitive cells in the bone marrow. This happens despite the presence in the peripheral blood of polymorphonuclear leukocytes with hypersegmented nuclei and occasional macroovalocytes, which testify to the fact that some megaloblastic hematopoiesis must be taking place, and despite subsequent confirmation of this diagnosis by determination of the serum vitamin B_{12} level[17] (which may often be in the "low normal" range) and the Schilling test. (See CHAPTER 6, "Clinical Picture," page 10 for cases in point.)

A recent report[44] calls attention to the possible usefulness of discriminating from megaloblasts lines of red cell development morphologically intermediate between unequivocal megaloblastic erythropoiesis and normoblastic erythropoiesis (i.e., intermediate megaloblasts).[8, 44, 4] The authors of that report believe that this modified type of megaloblastic erythropoiesis represents the morphologic result of the combination of a condition that would usually produce a megaloblastic marrow and an associated iron deficiency at the marrow level.

Actually, as eminent authorities[48] have noted, "intermediate megaloblasts" or "macroblasts" may be simply eryth

rocyte precursors with a supply of vitamin B_{12} or folic acid which is insufficient for normal maturation but not so grossly inadequate as to result in complete megaloblastosis They are characteristic of minor or partial deficiencies of vitamin B_{12} or folic acid Intermediate megaloblasts along with large but not quite giant metamyelocytes are often the only observable abnormality in the bone marrow of patients who are not yet severely anemic but who do have overt macroovalocytosis and an increase in the number of hypersegmented polymorphonuclear leukocytes in the peripheral blood The author has seen intermediate megaloblastic erythropoiesis in a number of cases of pure vitamin B_{12} or folic acid deficiency during the period of slight anemia which precedes the full blown clinical picture of megaloblastic anemia as well as early in the period of response to therapy No associated iron deficiency existed in these cases

In all 4 cases of the report mentioned [43] hypersegmented polymorphonuclear leukocytes and macrocytes were noted in the peripheral blood indicating the existence of an underlying megaloblastic erythropoiesis which was manifested in the bone marrow by intermediate megaloblastic erythrocyte development It is true that associated iron deficiency often results in an intermediate megaloblastic bone marrow morphology This is the product of interaction of the tendency toward megaloblastosis induced by folic acid or vitamin B_{12} deficiency and the tendency toward microcytosis induced by iron deficiency These cases [43] along with others in which evaluation of the bone marrow morphology may be difficult further confirm the significance of the presence in the peripheral blood of both macrocytes and hypersegmented polymorphonuclear leukocytes in increased numbers

The intermediate megaloblasts observed in combined iron and vitamin B_{12} (or folic acid) deficiency are readily converted to overt megaloblasts by iron therapy Conversely vitamin B_{12} *(or folic acid) therapy will convert the normochromic cells to* hypochromic cells

The presence in the peripheral blood of either macrocytes or

normal young polymorphonuclears with horseshoe or sausage shaped nuclei. More careful inspection reveals that what appears to be a single segment is in fact a rosette of overlapping segments or rather a sausage which is being pinched into a number of small round sausages. These abnormal young polymorphonuclears when in the bone marrow are the immediate precursors of the typical hypersegmented polymorphonuclear leukocytes usually seen in the peripheral blood.[4] (See FIGURE 3.)

When macroovalocytes and hypersegmented polymorphonuclear leukocytes are found in the peripheral blood, examination of the bone marrow obtained by aspiration biopsy should be the next step. Even if a megaloblastic marrow is not found in such circumstances, however, work-up of the patient for an incipient megaloblastic anemia will be fruitful in the large majority of cases. It cannot be emphasized too strongly that in cases of incipient megaloblastic anemia, even highly trained hematologists may not find clearly megaloblastic primitive cells in the bone marrow. This happens despite the presence in the peripheral blood of polymorphonuclear leukocytes with hypersegmented nuclei and occasional macroovalocytes, which testify to the fact that some megaloblastic hematopoiesis must be taking place, and despite subsequent confirmation of this diagnosis by determination of the serum vitamin B_{12} level[17] (which may often be in the "low normal" range) and the Schilling test. (See CHAPTER 6, "Clinical Picture," page 10, for cases in point.)

A recent report[43] calls attention to the possible usefulness of discriminating from megaloblasts "lines of red cell development morphologically intermediate between unequivocal megaloblastic erythropoiesis and normoblastic erythropoiesis (i.e., intermediate megaloblasts)."[4, 44] The authors of that report believe that this modified type of megaloblastic erythropoiesis represents the morphologic result of the combination of a condition that would usually produce a megaloblastic marrow and an associated iron deficiency at the marrow level.

Actually, as eminent authorities[46, 47, 154] have noted, "intermediate megaloblasts" or "macroblasts"[17] may be simply eryth-

rocyte precursors with a supply of vitamin B_{12} or folic acid which is insufficient for normal maturation but not so grossly inadequate as to result in complete megaloblastosis. They are characteristic of minor or partial deficiencies of vitamin B_{12} or folic acid. Intermediate megaloblasts along with large but not quite giant metamyelocytes are often the only observable abnormality in the bone marrow of patients who are not yet severely anemic but who do have overt macroovalocytosis and an increase in the number of hypersegmented polymorphonuclear leukocytes in the peripheral blood. The author has seen intermediate megaloblastic erythropoiesis in a number of cases of pure vitamin B_{12} or folic acid deficiency during the period of slight anemia which precedes the full blown clinical picture of megaloblastic anemia as well as early in the period of response to therapy. No associated iron deficiency existed in these cases.

In all 4 cases of the report mentioned[43] hypersegmented polymorphonuclear leukocytes and macrocytes were noted in *the peripheral blood indicating the existence of an underlying* megaloblastic erythropoiesis which was manifested in the bone marrow by intermediate megaloblastic erythrocyte development. It is true that associated iron deficiency often results in an intermediate megaloblastic bone marrow morphology. This is the product of interaction of the tendency toward megaloblastosis induced by folic acid or vitamin B_{12} deficiency and the tendency toward microcytosis induced by iron deficiency. These cases[43] along with others in which evaluation of the bone marrow morphology may be difficult further confirm the significance of the presence in the peripheral blood of both macrocytes and hypersegmented polymorphonuclear leukocytes in increased numbers.

The intermediate megaloblasts observed in combined iron and vitamin B_{12} (or folic acid) deficiency are readily converted to overt megaloblasts by iron therapy. Conversely, vitamin B_{12} (or folic acid) therapy will convert the normochromic cells to hypochromic cells.

The presence in the peripheral blood of either macrocytes or

normal young polymorphonuclears with horseshoe or sausage shaped nuclei. More careful inspection reveals that what appears to be a single segment is in fact a rosette of overlapping segments or rather a sausage which is being pinched into a number of small round sausages. These abnormal young polymorphonuclears when in the bone marrow are the immediate precursors of the typical hypersegmented polymorphonuclear leukocytes usually seen in the peripheral blood.[4] (See FIGURE 3.)

When macroovalocytes and hypersegmented polymorphonuclear leukocytes are found in the peripheral blood, examination of the bone marrow obtained by aspiration biopsy should be the next step. Even if a megaloblastic marrow is not found in such circumstances, however, work up of the patient for an incipient megaloblastic anemia will be fruitful in the large majority of cases. It cannot be emphasized too strongly that in cases of incipient megaloblastic anemia, even highly trained hematologists may not find clearly megaloblastic primitive cells in the bone marrow. This happens despite the presence in the peripheral blood of polymorphonuclear leukocytes with hypersegmented nuclei and occasional macroovalocytes, which testify to the fact that some megaloblastic hematopoiesis must be taking place, and despite subsequent confirmation of this diagnosis by determination of the serum vitamin B_{12} level[17] (which may often be in the low normal range) and the Schilling test. (See CHAPTER 6, Clinical Picture, page 10 for cases in point.)

A recent report[44] calls attention to the possible usefulness of discriminating from megaloblasts lines of red cell development morphologically intermediate between unequivocal megaloblastic erythropoiesis and normoblastic erythropoiesis (i.e., intermediate megaloblasts).[44] The authors of that report believe that this modified type of megaloblastic erythropoiesis represents the morphologic result of the combination of a condition that would usually produce a megaloblastic marrow and an associated iron deficiency at the marrow level.

Actually, as eminent authorities[4, 17, 4] have noted, intermediate megaloblasts or macroblasts[2] may be simply eryth

only a hypochromic microcytic anemia with no macroovalocytes present[13]

In the author's experience despite the severity of the iron deficiency and despite partial or complete obliteration of macro ovalocytes in the peripheral blood in the presence of vitamin B_{12} or folic acid deficiency *the increase in number of neutrophilic polymorphonuclear leukocytes with hypersegmented nuclei is not masked* and provides a clue to the coexistence of vitamin B_{12} or folic acid deficiency with the iron deficiency (See FIGURE 4)

The author has seen such patients (often pregnant or with a malabsorption syndrome) with concomitant iron deficiency and vitamin B_{12} deficiency often with associated folic acid



FIG 4—Typical hypersegmented polymorphonuclear leukocytes due to vitamin B_{12} deficiency in the peripheral blood of a patient who did not have macroovalocytosis (or even macrocytosis) because of the concomitant iron deficiency. In this case a normocytic normochromic anemia was present *due to a perfect cancelling out* by each deficiency of the erythrocyte morphology caused by the other deficiency. Serum vitamin B_{12} = [illegible]0 μμg/ml serum iron = 110 μg per cent Hematocrit = [illegible]8 per cent hemoglobin = 9.4 Gm/100 ml red cell count = 3.[illegible] million/cu mm MCV = 8[illegible] μ³ MCHC = 34. After vitamin B_{12} treatment serum iron fell to [illegible]6 μg per cent and hypochromic microcytic anemia appeared

an increased number of hypersegmented polymorphonuclear leukocytes alone is of less significance in diagnosing possible megaloblastic anemia, actual or incipient, than the presence of both together. Reticulocytes are large red cells, and an increased number of reticulocytes may convey an erroneous impression that macrocytosis is present. A variable number of macrocytes (and infrequently even some macroovalocytes) are part of the anisocytosis and poikilocytosis which accompany many diseases, especially those of a primarily hematologic nature. Macrocytosis is a frequent accompaniment of liver disease[11, 13] and aplastic anemia; these subjects will be discussed subsequently.

An increased number of polymorphonuclear leukocytes with hypersegmented nuclei is less commonly seen than are macrocytes in conditions other than those leading to a megaloblastic anemia, but they do appear with sepsis and widespread malignancy (especially the leukemias) and characterize the interesting familial entity of "constitutional hypersegmentation of the neutrophil nucleus."[48] When both macroovalocytes and hypersegmented polymorphonuclears in increased numbers are clearly present, an incipient or actual megaloblastic anemia is the most likely cause.

It may well be that the hypersegmented polymorphonuclear leukocytes very occasionally seen with sepsis and widespread malignancy are in fact products of folic acid deficiency at the bone marrow level caused by extremely rapid cell turnover.

The hematologic features of vitamin B_{12} or folic acid deficiency are frequently partly masked by coincident iron deficiency.[3, 48] Most frequently this masking takes the form of a *dimorphic* peripheral blood picture, with some red cells macrocytic and usually fully hemoglobinated but sometimes hypochromic, and others microcytic and hypochromic.[4, 33, 34] Sometimes the microcytes of iron deficiency appear to cancel out the macrocytes of vitamin B_{12} or folic acid deficiency, and the patient's peripheral blood picture is that of a normocytic normochromic or hypochromic anemia. If the iron deficiency is of sufficient severity, one may observe in the peripheral blood

only a hypochromic microcytic anemia with no macroovalocytes present.[13]

In the author's experience, despite the severity of the iron deficiency and despite partial or complete obliteration of macroovalocytes in the peripheral blood in the presence of vitamin B_{12} or folic acid deficiency, *the increase in number of neutrophilic polymorphonuclear leukocytes with hypersegmented nuclei is not masked* and provides a clue to the coexistence of vitamin B_{12} or folic acid deficiency with the iron deficiency (See FIGURE 4.)

The author has seen such patients (often pregnant or with a malabsorption syndrome) with concomitant iron deficiency and vitamin B_{12} deficiency, often with associated folic acid



FIG. 4.—Typical hypersegmented polymorphonuclear leukocytes due to vitamin B_{12} deficiency in the peripheral blood of a patient who did not have macroovalocytes (or even macrocytes) because of the concomitant iron deficiency. In this case a normocytic normochromic anemia was present due to a perfect cancelling out by each deficiency of the erythrocyte morphology caused by the other deficiency. Serum vitamin B_{12} = [illegible]0 μμg/ml; serum iron = 110 μg per cent; Hematocrit = [illegible]8 per cent; hemoglobin = 9.4 Gm/100 ml; red cell count = 3.2 million/cu mm; MCV = 8[illegible] μ³; MCHC = 3[illegible]. After vitamin B_{12} treatment, serum iron fell to [illegible]6 μg per cent and hypochromic microcytic anemia appeared.

an increased number of hypersegmented polymorphonuclear leukocytes alone is of less significance in diagnosing possible megaloblastic anemia actual or incipient than the presence of both together. Reticulocytes are large red cells and an increased number of reticulocytes may convey an erroneous impression that macrocytosis is present. A variable number of macrocytes (and infrequently even some macroovalocytes) are part of the anisocytosis and poikilocytosis which accompany many diseases especially those of a primarily hematologic nature. Macrocytosis is a frequent accompaniment of liver disease[13, 13a] and aplastic anemia these subjects will be discussed subsequently.

An increased number of polymorphonuclear leukocytes with hypersegmented nuclei is less commonly seen than are macrocytes in conditions other than those leading to a megaloblastic anemia but they do appear with sepsis and widespread malignancy (especially the leukemias) and characterize the interesting familial entity of constitutional hypersegmentation of the neutrophil nucleus.[48] When both macroovalocytes and hypersegmented polymorphonuclears in increased numbers are clearly present an incipient or actual megaloblastic anemia is the most likely cause.

It may well be that the hypersegmented polymorphonuclear leukocytes very occasionally seen with sepsis and widespread malignancy are in fact products of folic acid deficiency at the bone marrow level caused by extremely rapid cell turnover.

The hematologic features of vitamin B_{12} or folic acid deficiency are frequently partly masked by coincident iron deficiency.[3, 48a] Most frequently this masking takes the form of a *dimorphic* peripheral blood picture with some red cells macrocytic and usually fully hemoglobinated but sometimes hypochromic and others microcytic and hypochromic.[49, 38a] Sometimes the microcytes of iron deficiency appear to cancel out the macrocytes of vitamin B_{12} or folic acid deficiency and the patient's peripheral blood picture is that of a normocytic normochromic or hypochromic anemia. If the iron deficiency is of sufficient severity one may observe in the peripheral blood

3 *The Biochemical Basis of Megaloblastic Hematopoiesis*

Biochemical studies of megaloblasts have been directed in two main channels (1) quantitative chemical studies to determine the differences in content between megaloblasts and normoblasts and (2) studies of the biochemical mechanisms whereby the differences in content occur

It has been determined that the large amount of cytoplasm of the megaloblast contains considerably more ribonucleic acid (RNA) than the cytoplasm of the normoblast [9, 51] this is true at all stages of maturation and is most striking when hemoglobinization is occurring and RNA is almost gone from the normoblast but still persists in significant concentration in the megaloblast

The large nucleus of the megaloblast has been estimated to contain as much as double the amount of deoxyribonucleic acid (DNA) as the normoblast nucleus though not all workers agree that the DNA level of the megaloblast is so high [29] There is a fairly definite increase in the RNA DNA ratio of the megaloblast this is reflected in an increased uracil thymine ratio [53]

Feulgen stained smears of megaloblasts demonstrated their nuclear chromatin to be present in smaller particles than that of normoblasts [9]

With the demonstration that in the mammal uridine may be incorporated into thymidine [4] it was possible to conceive of cytoplasmic RNA as the principal source of material for nuclear DNA and further to suggest that any failure of utilization of RNA to provide material for DNA formation would lead to a persistence of cytoplasmic RNA resulting in macrocyte production In this schema any retardation of DNA synthesis to the twice resting-cell concentration required for mitosis would cause the marrow to become crowded with cells waiting to divide and slowly building up their DNA content while a large

deficiency in whose peripheral blood striking hypochromia was present along with an increase in the number of polymorphonuclear leukocytes with hypersegmented nuclei but with no macroovalocytosis. Presented with such a case, outstanding hematologists have ignored the hypersegmented polymorphonuclears (and the intermediate megaloblasts and large metamyelocytes in the bone marrow) and have made a diagnosis of uncomplicated iron deficiency anemia. These diagnoses were subsequently proved wrong by determination of the serum vitamin B_{12} level to be below 100 μμg/ml, the serum folic acid level to be below 5 mμg/ml, and development of overt megaloblastic anemia with macroovalocytosis after iron therapy and cure by administration of vitamin B_{12} (and folic acid when required). Deficiency of both iron and vitamin B_{12} without concomitant folic acid deficiency may be noted after total gastrectomy (and occasionally after distal subtotal gastrectomy), with terminal ileitis or other secondary malabsorption states, in elderly patients with histamine-refractory achlorhydria and anemia,[48] and as a result of two separate disorders coincidentally occurring in the same patient. An example of the latter is the coincidental occurrence of pernicious anemia and gastrointestinal tract bleeding.

Thus, in the presence of hypochromic erythrocytes in the peripheral blood, it is wise to suspect a concomitant megaloblastic anemia, even if no macroovalocytes are present, when one observes an increased number of hypersegmented polymorphonuclear leukocytes, especially when intermediate megaloblasts and large metamyelocytes are present in the bone marrow. It is possible that in conditions other than iron deficiency but also characterized by defective hemoglobin synthesis, macroovalocytes may also be absent despite the presence of a coincidental vitamin B_{12} or folic acid deficiency.

Even when the erythrocytes appear normochromic, it is wise to suspect vitamin B_{12} or folic acid deficiency with associated iron deficiency if there is an increased number of polymorphonuclear leukocytes with nuclei containing more than three lobes

molecules. Vitamin B_{12} influences folic acid metabolism,[72] but there is no evidence at present that folic acid directly influences vitamin B_{12} metabolism.

When a patient with a megaloblastic anemia is treated with vitamin B_{12} or folic acid, depending on his primary deficiency, the RNA/DNA ratio rapidly falls to normal. In pernicious anemia bone marrow cultures, vitamin B_{12} speeds up the production of DNA.[3] The speed of these phenomena explains the rapidity with which the megaloblastic marrow converts to normoblastic on institution of treatment; almost complete conversion *usually occurs within 24 to 48 hours. If conversion does not occur with the anticipated speed,* either complications are present or the wrong agent is being used. (Temporary responses may be obtained with folic acid in vitamin B_{12} deficiency states, or conversely vitamin B_{12} in folic acid deficiency states, but eventually the patient becomes refractory to the wrong agent, presumably as a result of complete exhaustion of his remaining stores of the vitamin in which he was deficient.)

Biochemical studies have done much in the way of delineating the mechanism of megaloblastic blood formation, but many questions still remain unanswered. It is presently accepted that folic acid deficiency leads directly to megaloblastic anemia; it is not yet certain that vitamin B_{12} deficiency is a direct cause of the disease. The question is still open as to whether the megaloblastic anemia which follows vitamin B_{12} deprivation is a direct result solely of such deprivation or is partly the result of *deranged folic acid metabolism caused by* deficiency of vitamin B_{12}.

In this connection, it is of interest that 5 to 10 mg of folic acid daily (10 to 20 times the rough minimal daily maintenance requirement) will usually produce hematologic remission in previously untreated patients with vitamin B_{12} deficiency, whereas as much as 1000 μg of vitamin B_{12} daily (perhaps 1000 times the rough minimal daily maintenance requirement) is *often inadequate to produce* hematologic remission in patients with primary folic acid deficiency.

backlog of RNA builds up in the cytoplasm. Biochemical evidence of blockage in pyrimidine nucleotide formation in vitamin B_{12} deficiency continues to be gathered.[58]

The megaloblast may thus be explained as a young red blood cell resulting from a prolonged phase between mitoses* allowing a longer time for dispersion of the chromatin throughout the nucleus,[...] thus producing its typical finely particulate appearance. Anything which retards the rate of cell division could therefore cause megaloblasts to be produced.

The concept of the megaloblast as a product of retardation of the nuclear evolution by blockage of the mitoses of maturation has been repeatedly stressed in the literature and recently thoroughly reviewed by Cazal[illegible] and by Reisner[illegible]. It would seem that any retardation of cellular proliferation would be manifested not only in the hematopoietic system but also in other areas where the production of new cells is normally rapid. Such indeed is the case, as demonstrated by the presence of megaloblastic oral, gastric and vaginal epithelial cells in patients with pernicious anemia.[3, 56, 8]

Similar alterations in gastric cytology have been described in pernicious anemia and in sprue.[3] Such similarities may be interpreted as the *effect* of inadequate absorption of vitamin B_{12} (and folic acid) and other deficiencies. Interpreting them as a *cause* of the inadequate absorption would be hazardous.

In practical clinical terms megaloblastic blood formation is observed nearly always in the presence of inadequate amounts of vitamin B_{12}, folic acid, or both. After the isolation of folic acid in 1943[61, 6] and vitamin B_{12} in 1948[63, 64] it was possible to study the role of these two agents in nucleoprotein synthesis.

Both of these agents function in the metabolism of one carbon units: folic acid primarily as a carrier of hydroxymethyl and formyl groups[6] and in formimino group transfer;[...] vitamin B_{12} in the *de novo* synthesis of labile methyl groups.[3] These vitamins are therefore essential in the synthesis of purines and pyrimidines,[68-...] which are integral parts of RNA and DNA.

*It should be noted that arrest of mitosis of cultured chick embryo ... of la... and fibroblasts induced by folic acid antagonists occurs in metaphase.

and kidney and sea food such as oysters and clams are especially rich in this vitamin Other dietary sources include all meats and sea food and to a lesser degree eggs milk and milk products [77 ...] Failure to ingest an adequate quantity of animal protein will eventually lead to vitamin B_{12} deficiency

A Vegans

Vegans are strict ovolacto vegetarians who subsist on a diet devoid of animal protein including dairy products and eggs The eventual development of vitamin B_{12} deficiency in such individuals has been documented by Wokes Badenoch and Sinclair [50] The vegan whose diet is adequate in folic and ascorbic acid content provides the best instance of pure nutritional vitamin B_{12} deficiency The other nutritional vitamin B_{12} deficiency states are associated *with multiple other dietary* deficiencies often including folic and ascorbic acids and it is difficult to determine to what extent megaloblastic anemia is caused by lack of folic and ascorbic acids in such cases (see C)

B Chronic Alcoholism

Prolonged alcoholism with a concomitant reduction in ingestion of vitamin B_{12} containing foods may result in overt vitamin B_{12} deficiency [51 53] When cirrhosis is associated with the chronic alcoholism the development of a megaloblastic anemia is more likely a reflection of folic acid deficiency than of vitamin B_{12} deficiency [54] (See section Cirrhosis Associated with Chronic Alcoholism page 52)

C Poverty Religious Tenets Ignorance Dietary Faddism

Poverty with ingestion of a diet containing very little animal protein such as the bread olives beans and tomatoes which made up the diet of the Macedonians studied by Fairley et al results in vitamin B_{12} deficiency along with multiple other deficiencies Abstention from animal protein on religious grounds will also produce vitamin B_{12} deficiency if the abstention is

4 *Etiologic Classification of the Megaloblastic Anemias Due to Vitamin B_{12} Deficiency*

As STATED IN THE PREFACE, the presence of a megaloblastic anemia is almost pathognomonic of a deficiency of vitamin B_{12}, folic acid, or both. Failure adequately to ingest, absorb, utilize, or retain either or both of these vitamins accounts for nearly every case of megaloblastic anemia. The small percentage of cases which have not yet been demonstrated to be a result of a deficiency in availability of either of these vitamins at the bone marrow level is almost uniformly characterized by inhibition or exhaustion of the bone marrow. It may well be that the megaloblastosis in these conditions will prove to be caused by interference with utilization or local exhaustion of the supply of vitamin B_{12} or folic acid at the bone marrow level.

Since deficiency of vitamin B_{12} or of folic acid directly or indirectly underlies nearly all, if not all, of the clearly megaloblastic anemias recorded in the literature at the present time, classification of these anemias in terms of etiology of the deficiency simplifies understanding, allows a methodological approach to differential diagnosis, and insures appropriate therapy.

I INADEQUATE INGESTION

The daily dietary requirement of the adult for vitamin B_{12} is approximately 1 μg. This assumption is based on the facts that a daily parenteral dose of 1 μg induces remission in pernicious anemia[74,75] and a similar amount of the vitamin provides a minimal maintenance dosage.[31,71,76]

Vitamin B_{12} is synthesized by microorganisms and is found in the tissue and fluid stores of marine and animal life which ingests such microorganisms. Therefore, the sources of vitamin B_{12} in the human diet are the protein-rich animal products. Liver

of utmost clinical significance, for it is interference with this pattern that produces the megaloblastic anemias resulting from failure to absorb vitamin B_{12}.

The other mechanism of absorption is independent of intrinsic factor but is primarily operative only in the presence of supraphysiologic doses of vitamin B_{12} (that is, amounts much greater than those found in the usual diet). A significant aliquot of such doses is absorbed by a mass action effect, possibly diffusion.[97,98] This mechanism has clinical significance primarily in oral therapy of vitamin B_{12} deficiency and will be discussed further in that connection.

A. Inadequate or Absent Secretion of Intrinsic Factor

The prototype of all the megaloblastic anemias is addisonian pernicious anemia, described as an etiological entity by Sir Thomas Addison in 1855[18] and Biermer in 1872.[14] Subsequent expansion of knowledge led to the realization that the megaloblastic anemias were not an etiologic entity but rather the similar clinical and pathologic result of various causes. At the present time the term "pernicious anemia" (or "addisonian pernicious anemia") is reserved in the Anglo-American literature for that megaloblastic anemia characterized by an idiopathic deficiency of Castle's intrinsic factor.[85] In other countries the term "pernicious anemia" is given a wider interpretation and includes all of the "pure" vitamin B_{12} deficiency states.[100]

Following hard on the heels of Minot and Murphy's Nobel prize winning discovery that a diet high in liver produced remission in 45 patients with pernicious anemia,[101] Castle and his associates[2-4] demonstrated that beef muscle (an animal protein) contained an "extrinsic (food) factor" which interacted with an "intrinsic factor" in normal human gastric juice to yield an "anti-pernicious anemia principle" which restored hematopoiesis to normal when given orally to the pernicious anemia patient. The oral administration of either extrinsic factor or intrinsic factor alone had no effect.

complete[13, 8] The author has observed pure nutritional vitamin B_1 deficiency in a 20 year old Columbia University student from India who ate no animal protein for religious reasons He had megaloblastic anemia a serum vitamin B_1 level of 19 $\mu\mu$g/ml (L gracilis) a normal serum folic acid level of 8 mμg/ml (L casei) and no urinary excretion of formiminoglutamic acid

A similar phenomenon may sometimes be observed in elderly persons who live alone and eat little except for easy to prepare carbohydrate foods[86] and in certain dietary faddists[87]

However all of the patients of whom the author is aware who had megaloblastic anemia associated with *grossly* inadequate diets had folic acid deficiency manifested usually with normal serum vitamin B_1 levels and very low serum folic acid levels (See section Nutritional Megaloblastic Anemia page 51)

D Hyperthyroidism

Hyperthyroidism has been reported in association with megaloblastic anemia in two series of cases[88, 89] It is possible this association may be a consequence of increased demand for and turnover of vitamin B_1 in thyrotoxicosis[10, 91] in the presence of a marginal dietary intake especially since the anemia may disappear after subtotal thyroidectomy[9] The concomitant requirement of marginal intake for vitamin B_1 deficiency to develop would explain the low incidence of megaloblastic anemia among thyrotoxic patients

II DEFECTIVE ABSORPTION

The majority of cases of megaloblastic anemia due to vitamin B_1 deficiency fall into the various categories of this classification

There appear to be two separate and distinct mechanism for absorption of vitamin B_1[93] With physiologic doses of vitamin B_1 (that is amounts of 0 to 2 μg is found in the average American meal[illegible]) absorption is dependent upon intrinsic factor[97] This normal pattern of absorption is the one

deficiency[127] has revealed that with the vitamin B_{12} deficiency a variable degree of gastric atrophy develops and a variable degree of reversion of the histologic picture of the stomach toward normal accompanies the therapy. This would suggest that in some instances vitamin B_{12} deficiency may be the cause rather than the result of gastric atrophy. Prolonged iron deficiency may also cause gastric atrophy[133] eventuating in loss of intrinsic factor secretion and addisonian pernicious anemia.[129]

Addisonian pernicious anemia is itself probably not an etiologic entity but rather a syndrome of several possible etiologies which will be detailed below.

(1) *Hereditarily determined failure of intrinsic factor secretion.* The physiologic mechanism responsible for stimulating gastric secretion of intrinsic factor is unknown. Inadequate functioning of this mechanism or of the equally unknown enzymatic mechanism of production of intrinsic factor is responsible for the most widely studied form of pernicious anemia, with the possible exception of that anemia resulting from degenerative gastric atrophy.

Hereditarily determined primary failure of gastric secretion of intrinsic factor has been inferred from the rare cases of pernicious anemia in childhood,[15, 130] pernicious anemia without achlorhydria,[131, 133] the high association coefficient of these two types of pernicious anemia, the high familial tendency of the disease,[134, 138] and its occurrence in monovular twins.[137]

Harris, Jones, Swan, and Tudhope[135] have reported the case of a 16 year old girl with megaloblastic anemia whose baseline secretion of intrinsic factor was inadequate. Parenteral administration of the powerful secretagogue and parasympathetic stimulant Carbachol (carbamylcholine chloride) induced secretion of a normal quantity of intrinsic factor. Previously, Mollin, Baker, and Doniach[134] had reported a similar case in a young man; in their case the deficient baseline secretion of intrinsic factor was only partly correctible by administration of Carbachol.

In some individuals the usual secretion of intrinsic factor under physiologic circumstances is low, though these individuals

Two decades later vitamin B_1 was demonstrated to be the extrinsic factor[106] and also to be the anti pernicious anemia principle[107] Since vitamin B_1 is the anti pernicious anemia principle and is fully effective in treating pernicious anemia when administered parenterally the role of intrinsic factor appears to be that of effecting the transfer of ingested vitamin B_1 across the intestinal mucosa and into the blood stream Whether the vitamin B_1 is absorbed alone or the vitamin B_1 intrinsic factor combination is absorbed has not yet been determined Favoring the latter possibility is a report of the presence in human serum of a substance similar to intrinsic factor[108, 109] Furthermore there is some experimental evidence that receptors for intrinsic factor may exist in both intestinal mucosa[108, 11] and liver[109–110] and that intrinsic factor may be involved in the selective deposition of vitamin B_1 in the liver[108, 110] This work requires confirmation and extension before general acceptance

The chemical nature of intrinsic factor is not known since this substance has not been isolated in pure form It is probably a mucoprotein or mucopolypeptide[116–11] In man the glandular structure of the fundus and cardiac portions of the stomach is the site of intrinsic factor secretion[1 0 1 1] In the rat the chief (pepsinogen) cells of the fundus glands probably are the intrinsic factor elaborating cells[1]

1 Failure of the Stomach to Adequately Secrete Intrinsic Factor

a *Addisonian Pernicious Anemia*

This term includes all of the megaloblastic anemias of vitamin B_1 deficiency resulting from inadequate secretion of intrinsic factor in which the basic cause of this deficient secretion is unknown In these cases the gastric mucosa rarely may be histologically normal but usually shows gastric atrophy or an atrophic gastritis of varying severity[1 3 1 6]

Extensive study of patients with fish tapeworm vitamin B_1

longed iron[28] or vitamin B_{12}[27] deficiency may produce gastric atrophy that atrophy passes through stages of gastritis before being established Serial studies of the gastric mucosa in large numbers of living patients will be needed to clarify these relationships

b *Lesions Which Destroy the Gastric Mucosa*

Ingested corrosives[29] linitis plastica of non neoplastic origin[33] and extensive neoplastic disease[1] may each destroy the intrinsic factor secreting glandular lining of the stomach and thereby eventually produce a megaloblastic anemia due to vitamin B_{12} deficiency

Neoplastic disease producing megaloblastic anemia must be separated from gastric carcinoma arising in patients with pernicious anemia The incidence of gastric carcinoma in autopsied patients with both pernicious anemia and gastric atrophy is about 10 to 12 per cent this is more than three times the expected occurrence of gastric carcinoma at autopsy[131] The high coincidence of gastric carcinoma and pernicious anemia is probably a consequence of the fact that both conditions frequently arise from a common genetically determined gastric atrophy[144, 24] or perhaps that the gastric atrophy which results in pernicious anemia is also associated with intestinal metaplasia in the body area of the stomach[1] which may be a precancerous lesion In a given patient with carcinoma of the fundus or body of the stomach with gastric atrophy and pernicious anemia it is impossible at present to determine *post hoc* which came first

c *Endocrine Disorders*

Pituitary and gonadal insufficiency have been reported to produce infrequently a pernicious anemia like picture leading to the suggestion that a hormonal inadequacy may lead to degeneration of the intrinsic factor producing cells[16]

In the few cases of hypothyroidism in which a megaloblastic anemia has appeared and causes of megaloblastic anemia unrelated to hypothyroidism have been excluded it has appeared

may have normal serum vitamin B_1 levels and histologically normal gastric mucosa. Carbachol stimulation evoked normal secretion of intrinsic factor in a group of such subjects, but one of them was eventually unable to secrete intrinsic factor even after such stimulation and was then classified as a case of pernicious anemia.[139,140] A possibly similar such case is now under observation.[141]

Study of patients diagnosed as having pernicious anemia in the 1930's revealed that many had small amounts of intrinsic factor in their gastric juice.[14] It is not possible as yet to delineate the exact etiology of the megaloblastic anemia resulting from idiopathic (and most probably hereditary[143,144]) failure of secretion of intrinsic factor; the evidence at present indicates it results from an enzymatic defect in the production of intrinsic factor, resulting in inadequate production and eventual total cessation of production. Primary inadequacy of the *stimulus* to intrinsic factor secretion has not yet been excluded as an etiologic factor, however.

(2) *Hereditarily determined degenerative gastric atrophy.* A genetically determined tendency to develop gastric atrophy with aging has been well documented,[143,145] as has the frequent (but not uniform) association of gastric atrophy and failure of intrinsic factor secretion.[1,6,143,147] The possible relation of this entity to (1) above and (3) below is undetermined, but its frequency of occurrence is high.

(3) *Gastric atrophy as the end stage of superficial inflammatory gastritis, superficial gastritis with atrophy.* Pernicious anemia has been observed in gastric atrophy resulting from chronic gastritis[148] and associated with superficial gastritis with atrophy, atrophic gastritis of varying severity, and gastric atrophy in which relationship to gastritis was not established.[1,14] Whether these different types of lesions in adult pernicious anemia are stages in the same pathological process and closely related to (1) and (2) above, or distinct etiologic entities falling into separate categories of atrophic gastritis and gastric atrophy, remains to be seen.[1] It has already been mentioned that pro

Previously[161] 5 patients had been reported with megaloblastic anemia developing from 2 to 12 years after subtotal gastrectomy accompanied by inadequate vitamin B_{12} absorption correctible by oral administration of intrinsic factor Of these 5 patients 4 had atrophy of the gastric mucosa as demonstrated by examination of a gastric biopsy despite the fact that such atrophy was not present at the time of gastrectomy The authors of that report noted that the patients were originally operated upon because of gastric ulcer which may itself have led to gastritis and eventual atrophy with loss of intrinsic factor secretion

Loewenstein[163] noted low vitamin B_{12} absorption in 3 of 22 patients who had distal subtotal gastrectomy All 3 patients were tested for B_{12} absorption ability more than 6 years after operation whereas most of the other 19 patients were tested less than 6 years after operation None of the 3 patients with low B_{12} absorption had macrocytic anemia in fact 2 had hypochromic microcytic anemia associated with a low serum iron concentration i e iron deficiency anemia All 3 had gastro*jejunostomies* after their gastric resection

Still more recently[50] decreased absorption of vitamin B_{12} correctible by oral administration of intrinsic factor was found in 4 of 19 patients who had had subtotal gastrectomy followed by gastro*jejunostomy* but in none of 15 patients who had had subtotal gastrectomy followed by gastro*duodenostomy* None of the 34 subjects had anemia The surgical specimens at gastrectomy of 3 of the 4 patients with subsequently decreased vitamin B_{12} absorption had normal gastric mucosa

Putting these various findings together it seems that following distal subtotal gastrectomy especially if it is followed by gastrojejunostomy rather than gastroduodenostomy gastric secretion of intrinsic factor may be diminished[16] In the course of a number of years atrophy may develop in the gastric remnant with loss of residual ability to secrete intrinsic factor[164] Mild vitamin B_{12} deficiency may occur not infrequently but severe megaloblastic anemia as a direct result of the distal

to result from defective intestinal absorption of vitamin B_1 unrelated to secretion of intrinsic factor [157] However there may be a depressed secretion of intrinsic factor in some cases [157, 158]

Addison s disease has also on rare occasions been reported in association with pernicious anemia [159, 160] The relation of these two conditions is obscure

2 Gastrectomy

a *Total*

Total gastrectomy removes the source of intrinsic factor [161] and will invariably eventually produce megaloblastic anemia due to vitamin B_1 deficiency if appropriate prophylaxis with vitamin B_1 is not instituted [30] The period of time which will elapse before development of anemia will depend on the amount of the body stores of vitamin B_1 and on the dietary content of vitamin B_1 and folic acid

b *Subtotal*

(1) *Proximal* Proximal subtotal gastrectomy with esophagoantrostomy may ablate the intrinsic factor secreting glands to such an extent as to produce vitamin B_1 deficiency [162] Less extensive proximal subtotal gastrectomy is seldom followed by inadequate intrinsic factor secretion [163]

(2) *Distal* Distal subtotal gastrectomy is performed much more frequently than proximal subtotal gastrectomy In a survey of over 1500 patients who had this operation performed MacLean [164] found that only 9 patients subsequently developed a megaloblastic anemia associated with a hemoglobin below 10 Gm / 100 ml In every one of these 9 patients histologic review of the resected portion of the stomach demonstrated a gastric atrophy This suggested that these 9 patients would eventually have developed a megaloblastic anemia due to lack of intrinsic factor even if subtotal gastrectomy had not been performed and that the subtotal gastrectomy had no relation to the development of the megaloblastic anemia

and very high urinary uropepsin levels The anemia responded to intramuscular injections of vitamin B_{12} but not to oral administration of the vitamin together with intrinsic factor Radioactive vitamin B_{12} studies demonstrated extremely poor intestinal absorption not influenced by addition of intrinsic factor normal gastric juice or bowel sterilization with tetracycline Since five hundred ml of the patient s gastric juice plus vitamin B_{12} and intrinsic factor given orally to a woman with a subtotal gastrectomy and with normal B_{12} absorption depressed her absorption of B_{12} by 50% the authors concluded that this patient s gastric juice in sufficient quantity will inhibit the absorption of B_{12} from the intestinal tract causing a variant of pernicious anemia

However there is evidence that large amounts of intrinsic factor given orally such as the 500 ml of gastric juice plus additional intrinsic factor in the form of a hog intrinsic factor concentrate which was given to the woman with a subtotal gastrectomy may inhibit the absorption of vitamin B_{12} in any recipient [11, 169, 173] Therefore production of inhibited absorption with such a large dose of intrinsic factor-containing material does not constitute an argument in favor of the existence of an intrinsic factor inhibitor in the gastric juice that was given Furthermore if such an inhibitor did exist it might be expected that it could be overcome (although this is not necessarily true) by a large dose of intrinsic factor The authors report however that massive dosage of intrinsic factor given orally did not correct the absorptive defect

The validity of the contention that the patient s gastric juice contains an intrinsic factor inhibitor cannot yet be considered proved A mild form of coeliac disease or a selective malabsorption for vitamin B_{12} remain major diagnostic possibilities A search for evidences of malabsorption for other agents remains to be reported as does the result of oral administration of a *moderate* amount (25 to 100 ml) of the patient s gastric juice with radioactive vitamin B_{12} to a recipient with known addi

subtotal gastrectomy uncomplicated by other factors is rare A complicating factor might be the occasional development of a malabsorption syndrome after partial gastrectomy[16a] In this connection there is a greater rapidity of gastric emptying and intestinal transit after subtotal gastrectomy This is more marked after a Billroth II procedure (gastro*jejunostomy*) than after a Billroth I operation (gastro*duodenostomy*)[16b]

If the absorption of iron occurs chiefly in the duodenal region[13,166,166a] iron deficiency should be expected to occur more frequently when subtotal gastrectomy is followed by gastrojejunostomy than when it is followed by gastroduodenostomy Long continued iron deficiency itself may then lead to gastric atrophy and intrinsic factor deficiency[18,19,167]

When any patient undergoes subtotal gastrectomy the extent of the gastric remnant should be clearly and precisely recorded as should the appearance of the mucosa of the resected portion The patient must be closely checked for the possible development of iron deficiency His ability to absorb a test dose of radioactive vitamin B_1 should be determined about two months after operation[16] If poor and correctible by oral administration of intrinsic factor the patient should be treated for the rest of his life just as if he had pernicious anemia If his ability to absorb vitamin B_1 is normal the patient should have semiannual examinations of his peripheral blood smear and determination of his hematocrit hemoglobin and red cell count along with serum iron and vitamin B_1 determinations if available It is probably in the patient s best interest to continue such semiannual examinations for at least 10 years after the subtotal gastrectomy As stated previously however the incidence of megaloblastic anemia after subtotal gastrectomy is quite low even in patients followed for a number of years

3 Intrinsic Factor Inhibitor in Gastric Secretion

A recent preliminary report[168] presented an unusual case of pernicious anemia diagnosed in a male at the age of 13 months with a 25 year follow up The patient consistently had free acid

as proved by direct instillation of vitamin B_{12} into the colon[1,4] and the fact that the stools of patients with pernicious anemia contain large amounts of vitamin B_{12} synthesized by bacteria in the colon which would produce remission of the anemia if they could be absorbed[18] presumably a similar bacterial flora producing vitamin B_{12} is present in the stools of vegans who secrete intrinsic factor but still develop vitamin B_{12} deficiency

Since the small intestine provides the only physiologically significant absorptive surface for vitamin B_{12} it is clear why any derangement in this portion of the alimentary canal may produce vitamin B_{12} deficiency disease if the derangement is of sufficient chronicity so that the body stores of available vitamin B_{12} are exhausted

Study of individuals who had undergone total gastrectomy indicate that such stores become depleted to a sufficient extent to produce a macrocytosis in approximately one or two years with anemia appearing in another one or two years and a megaloblastic marrow shortly thereafter[36] The major site of storage of vitamin B_{12} appears to be the liver[3,18] in which the biologic half life of vitamin B_{12} is approximately a year[37]

In most patients with a small intestinal disorder of sufficient degree to produce defective absorption of vitamin B_{12} oral administration of supplementary intrinsic factor is not able to improve the absorption[189,191]

It is not easy to predict the time of onset of overt clinical vitamin B_{12} deficiency in any patient with an absorptive defect because the amount of the body stores the dietary intake and the metabolic turnover of vitamin B_{12} varies from person to person In addition failure to absorb vitamin B_{12} is usually not absolute and varies from time to time in the same individual

1 Malabsorption Syndrome

Malabsorption syndrome is a term applied to a group of metabolic disorders in which multiple defects of intestinal absorption play a prominent part[1,4,191a]

sonian pernicious anemia both in the absence and in the presence of added hog intrinsic factor concentrate

Nevertheless the speculation that some individuals may have an inhibitor of intrinsic factor in their gastric juice is interesting and worth keeping in mind There is some evidence that such an inhibitor may exist in the form of degraded or incomplete intrinsic factor unable to bind vitamin B_1 but able to attach to intestinal receptors and block their uptake of intrinsic factor [1 3]

B Small Intestinal Disorder

Variable degrees of reduced absorption of vitamin B_1 and of folic acid may occur with any structural or functional disorder affecting the small intestine

The absorption of physiologic amounts of vitamin B_1 occurs primarily in the small intestine This has been demonstrated by instillation of radioactive vitamin B_1 through an intestinal tube directly into the duodenum jejunum and ileum [1 4] It s probable that no significant absorption occurs from the stomach since oral administration of normal gastric juice with vitamin B_1 to gastrectomized patients is not only effective but may actually produce greater vitamin B_1 absorption than that observed on similar treatment of patients with pernicious anemia whose stomachs are intact [1] Bypassing the stomach by duodenal intubation [174] or timed release capsules [176] has also resulted in greater vitamin B_1 absorption than that observed when the stomach was not bypassed Possible explanations for this phenomenon include binding of vitamin B_1 by materials in the stomach that have no intrinsic factor activity [119 1 3 177 1] and the adverse affect of acid pH on vitamin B_1 absorption [11 150]

It is probable that most vitamin B_1 absorption in man occurs in the ileum [140 181 18] as it does in the rat [15 18] and the dog [186] There is practically no absorption of vitamin B_1 by patients who have had all their small intestine except the jejunum resected [140 181]

No significant absorption of vitamin B_1 occurs from the colon

cortical steroids [97] The mechanism of this correction is unknown Response of megaloblastic anemia to administration of adrenocortical steroids has been observed in some cases of adult coeliac disease and of pernicious anemia [14, 1] here too the mechanism of action of the steroids in terms of vitamin B_{12} (or folic acid) absorption is unknown

Permanent correction of the absorptive defect usually follows effective treatment of the folic acid deficiency which results in regeneration [0] of the intestinal villi [03] if the damage has not persisted for such a prolonged period as to be irreversible and generally a complete recovery of the patient [04, 16, 17]

For these reasons and because tropical sprue does not respond to a gluten free diet and often demonstrates a return of the folic acid excretion test to normal with treatment [04] tropical sprue is considered here as it is by many authorities [143, 03, 04, 17, 18] to differ from nontropical sprue In the latter condition the villi do not regenerate [03] actual cure does not occur [19] the patient often responds to a gluten free diet [19, 19a] and the folic acid excretion test remains abnormal after treatment [04] It should be noted however that some investigators believe that the dissimilarities are too superficial to warrant definition of tropical and nontropical sprue as separate entities [0, 1]

(2) *Childhood coeliac disease adult coeliac disease (idiopathic steatorrhea nontropical sprue)* These conditions are believed to be the expression of the same basic defect at different ages [04, 18] The villous atrophy is irreversible [03, 1] and cure is not yet possible [19, 4]

This entity is probably the final result of a hereditary constitutional defect in metabolism transmitted as a dominant trait of variable penetrance and expressivity [14, 18, 6, 9] It has been stated that pernicious anemia is the final result of a genetically determined progressive atrophy of the chief cells of gastric mucosa while coeliac disease and idiopathic steatorrhoea result from an analagous atrophy of the crypt cells of the intestinal mucosa [03]

a *Primary*

Primary malabsorption syndrome includes those generalized absorption defects of unknown etiology in which varying degrees of atrophy of the intestinal villi are present [00-05] The possible existence of receptors for intrinsic factor in the small intestine [111, 11, 06] suggests a direct relationship between the degree of damage of the intestinal villi (with concomitant reduction in number of receptors) and the degree of inability to absorb vitamin B_{12} A similar relationship may exist for folic acid absorption The variable degree and reversibility of the atrophy of the intestinal villi may explain in part the similar variable degree and reversibility of the absorptive defect for vitamin B_1 [190, 191, 0, 08]

Another factor which must be considered in evaluating the absorptive defect for vitamin B_1 in both primary and secondary malabsorption syndromes is the dependence of intrinsic factor action on calcium [112] Removal of calcium by fatty acids in the form of insoluble soaps may explain the poor vitamin B_1 absorption in some patients with steatorrhea [09] although in one series [1, 8] of 10 such patients peroral administration of 7 to 10 Gm of calcium lactate had no significant effect on absorption of concurrently administered radio B_1

The various conditions forming the group characterized as the *primary malabsorption syndrome* are differentiated from each other on clinical grounds such as age place of residence severity response to treatment and reversibility of the disease [19]

Although the primary malabsorption states frequently are accompanied by considerably impaired absorption of vitamin B_1 they are generally more intimately related to folic acid deficiency and will be discussed further in that connection

(1) *Tropical sprue* This steatorrhea syndrome seen in the tropics is probably most often the result of prolonged dietary deficiency of folic acid [10, 1] The associated absorptive defect for vitamin B_1 is sometimes correctible by maintaining the patient on adrenocorticotrophic hormone (ACTH) [13] or adreno

(5) *Other conditions characterized by chronically disturbed intestinal function* Macrocytic anemia has been reported in such conditions associated with chronic intestinal dysfunction as chronic pancreatic disease [39] chronic dysentery [40] hypothyroidism [1 7] and mesenteric vascular disease [49] In the latter two conditions resolution of the metabolic disorder and healing of the intestinal lesion respectively were associated with a return to normal of the intestinal absorption of vitamin B_1 which had been previously demonstrated to be greatly reduced There is some evidence in rats that the absorptive mechanism in the ileum for vitamin B_1 is slowed down by hypothyroidism [41] Decreased secretion of intrinsic factor may also occur with lack of thyroid hormone

2 Specific Malabsorption for Vitamin B_1

a *Due to Long Term Ingestion of Calcium Chelating Agents*

The mechanism of intrinsic factor action in the intestine appears to be calcium dependent [111 11 4] The amount of radioactive vitamin B_1 excreted in the urine of normal subjects during the course of a Schilling test after short term administration of calcium chelating agents such as sodium phytate or ethylene diamine tetraacetate (EDTA) is considerably decreased [06 43] In some patients with steatorrhea oral administration of calcium lactate increases the otherwise small amount of vitamin B_1 absorbed [09] In most cases it has no effect however [1 8]

Five patients receiving orally administered sodium phytate for periods from 19 to 44 months as treatment for hypercalcuria associated with renal stones [44] were investigated through the courtesy of Dr P H Henneman They were all found to have decreased serum vitamin B_1 levels increasingly marked in direct proportion to the length of therapy macroovalocytosis an increased amount of hypersegmentation of the nuclei of the polymorphonuclear leukocytes and diminished position and/or vibration sense in the toes [06 4]

b *Secondary*

Secondary malabsorption syndrome includes those clinical entities which produce gross pathologic alteration in the structure or function of the small intestine of sufficient degree and duration to result in deranged absorptive capacity The degree of the derangement is a major factor in determining whether vitamin B_{12} (and folic acid) deficiency will be produced

(1) *Regional ileitis* Megaloblastic anemia is a not infrequent result of this condition [30] which frequently produces grossly deficient vitamin B_{12} absorption In one study 42 patients out of 80 had low serum vitamin B_{12} levels and the impaired absorption could almost always be restored to normal by steroid therapy [9]

(2) *Strictures or anastomoses of the small bowel* Megaloblastic anemia has been observed to follow the development of single or multiple small bowel strictures usually in the ileum and intestinal anastomoses [31, 3] The latter have included entero enterostomies enterocolostomies gastrojejunocolic fistulae and gastrocolic fistulae

Defective absorption of vitamin B_{12} or folic acid or both is demonstrable in a significant percentage of such patients [1] However in the majority of such patients when macrocytic anemia develops it appears to be the result of intestinal stasis producing an abnormal bacterial flora and the blind loop syndrome rather than a secondary malabsorption syndrome [231, 13]

(3) *Intestinal resection* Intestinal resections as performed for jejunoileitis or superior mesenteric artery occlusions may result in failure adequately to absorb vitamin B_{12} [191, 34]

(4) *Malignancies and granulomatous lesions involving the small intestine* Lymphosarcoma reticulum cell sarcoma lymphogranulomatosis and intestinal lipodystrophy are among the conditions which may produce a malabsorption syndrome with compromise of vitamin B_{12} absorption to a variable degree [3, 48]

(5) *Other conditions characterized by chronically disturbed intestinal function* Macrocytic anemia has been reported in such conditions associated with chronic intestinal dysfunction as chronic pancreatic disease [39] chronic dysentery [40] hypothyroidism [1 7] and mesenteric vascular disease [08] In the latter two conditions resolution of the metabolic disorder and healing of the intestinal lesion respectively were associated with a return to normal of the intestinal absorption of vitamin B_{12} which had been previously demonstrated to be greatly reduced There is some evidence in rats that the absorptive mechanism in the ileum for vitamin B_{12} is slowed down by hypothyroidism [41] Decreased secretion of intrinsic factor may also occur with lack of thyroid hormone

2 Specific Malabsorption for Vitamin B_{12}

a *Due to Long Term Ingestion of Calcium Chelating Agents*

The mechanism of intrinsic factor action in the intestine appears to be calcium dependent [111 11 4] The amount of radioactive vitamin B_{12} excreted in the urine of normal subjects during the course of a Schilling test after short term administration of calcium chelating agents such as sodium phytate or ethylene diamine tetraacetate (EDTA) is considerably decreased [42 43] In some patients with steatorrhea oral administration of calcium lactate increases the otherwise small amount of vitamin B_{12} absorbed [09] In most cases it has no effect however [1 8]

Five patients receiving orally administered sodium phytate for periods from 19 to 14 months as treatment for hypercalcuria associated with renal stones [44] were investigated through the courtesy of Dr P H Henneman They were all found to have decreased serum vitamin B_{12} levels increasingly marked in direct proportion to the length of therapy macroovalocytosis an increased amount of hypersegmentation of the nuclei of the polymorphonuclear leukocytes and diminished position and/or vibration sense in the toes [06 4]

Examination of the bone marrow morphology in each of these 5 patients revealed the presence of some intermediate megaloblasts and some large but not quite giant metamyelocytes.

The inference from these studies would seem to be that patients receiving prolonged oral therapy with oral calcium chelating agents will eventually develop a full blown megaloblastic anemia due to vitamin B_{12} deficiency if not prophylactically treated by injection of the vitamin at regular intervals. However, absorption of radioactive vitamin B_{12} in 2 μg doses by the 5 patients on prolonged phytate therapy appeared to be normal.[91] Since it was not determined that the radioactivity absorbed was that of the vitamin B_{12} rather than that of a breakdown product of the vitamin and since other possibilities also could explain the discrepant results, further study is needed to determine clearly whether or not long term ingestion of calcium chelating agents will sufficiently restrict B_{12} absorption eventually to cause full blown megaloblastic anemia. All the evidence, except for the normal absorption of radioactive vitamin B_{12}, suggests that overt megaloblastic anemia will in fact eventually develop in such cases.

b *Due to Absence of Intestinal Receptors for Intrinsic Factor*

According to a recent hypothesis[110, 11] which coincides with the views of various workers[113, 11, 11] receptors for intrinsic factor exist in the small intestine and play a vital role in the absorption of physiologic amounts of vitamin B_{12}. It would be logical to suspect that if such receptors exist, patients might be found who are unable to absorb vitamin B_{12} because of a congenital or acquired reduction or absence of these receptors independent of any other absorptive defects.

In a search for such patients the author has seen 7 individuals with megaloblastic anemia, serum vitamin B_{12} levels below 100 $\mu\mu$g/ml, inability to absorb a 2 μg oral dose of radioactive vitamin B_{12} either alone or with added hog intrinsic factor concentrate, intrinsic factor secretion appearing to be

normal as measured by in vitro assay of gastric juice and no steatorrhea diarrhea or other signs or symptoms of malabsorption syndrome

Two of these patients developed steatorrhea during fat balance studies and were classified as cases of adult coeliac disease

A third B C a 70 year old woman of Puerto Rican origin was extensively studied at the Mount Sinai Hospital Examination included a full battery of studies for possible malabsorption of agents other than vitamin B_{12} by Dr D Adlersberg s group Glucose fat vitamin A and xylose tolerance tests fat balance studies gastrointestinal tract roentgenograms and fluoroscopy plasma prothrombin and serum calcium phosphorus alkaline phosphatase and folic acid were all normal

This woman was one of the patients we made available to Drs Luhby Cooperman and Teller for their studies using their method of histidine loading[511] they found no biochemical folic acid deficiency (as manifested by excretions of formiminoglutamic acid in the urine which were well within the normal range after many repeated histidine loading doses)

There was free acid in her gastric juice after but not before histamine stimulation The only suggestion of an absorptive abnormality other than for vitamin B_{12} was a low serum carotene level of 51 µg per cent This could have been explained on the basis of her poor diet[61] but it could also be a reflection of an otherwise completely hidden malabsorption syndrome

The fourth patient M C was studied at the Mount Sinai Hospital almost exactly as patient B C (*vide supra*) This 71 year old woman of Puerto Rican origin had a serum carotene level of 13 µg per cent and a serum folic acid level of 2 4 mµg ml indicative of folic acid depletion despite the absence of formiminoglutamic acid in her urine (histidine loading was not done in this case) All other studies yielded normal results While her low serum carotene could be explained by her poor diet it is harder to explain the low serum folic acid level She is being further studied to delineate more clearly the condi

tion The present working diagnosis is malabsorption syndrome but dietary folic acid deficiency plus a specific absorptive defect for vitamin B_1 due to lack of intestinal receptors for intrinsic factor remains an alternate possibility

The final 3 patients have been partially studied for absorptive defects other than for vitamin B_1 including three day fat balance studies with no such defects found This was also true of other cases alluded to in the literature [173, 4]

One or more of the 5 above noted cases may eventually be definitively proved to have solely a specific absorptive defect for vitamin B_1 due to a reduction or absence of intestinal receptors for intrinsic factor or of whatever other small intestinal mechanism or mechanisms which may be involved in vitamin B_1 absorption In all of these 5 cases repetitive studies at varying intervals of time are required to rule out an occult generalized malabsorption syndrome whose various other parameters may be only intermittently demonstrable Other cases of possible specific malabsorption for vitamin B_1 are being closely followed elsewhere [4]

At present it may be said that specific malabsorption for vitamin B_1 does exist but its existence as an entity independent of adult coeliac disease (and possibly other etiologic entities in the group of generalized malabsorption syndromes) cannot be considered proved until extensive longitudinal studies over a period of years have been carried out or until jejunal biopsies are obtained

It must be remembered that some patients with malabsorption syndrome may not demonstrate malabsorption for fats even on serially repeated studies and may show malabsorption only for glucose on one occasion only for vitamin A on another occasion etc

Unfortunately none of our five patients had jejunal biopsy Demonstration of a normal jejunal mucosa in these individuals would effectively exclude idiopathic steatorrhea and help to establish definitively the diagnosis of specific malabsorption for vitamin B_1

C Competition for Vitamin B_{12} by Intestinal Parasites or Bacteria

1 Fish Tapeworm (Diphyllobothrium Latum)

Next to addisonian pernicious anemia fish tapeworm anemia is probably the most thoroughly investigated of all the vitamin B_{12} deficiency states[100] It has been directly demonstrated both in vitro[46] and in vivo[47] that the fish tapeworm competes directly with the intrinsic factor of the host for available vitamin B_{12} in the intestine[48]

The higher the parasite is located the more likely it is to interfere with vitamin B_{12} absorption[49] since the vitamin has less prolonged contact with the intestine and therefore less chance of being absorbed prior to being taken up by the tapeworm

Other factors influencing the relative uptake of vitamin B_{12} by the parasite as compared to the host are the number and size of the worms[100, 47] the rapidity of growth and of egg production by the worms[47] and the quantity of intrinsic factor produced by the host[48] Vitamin B_{12} bound to intrinsic factor is unavailable to the parasite[46] but is available to the host

Vitamin B_{12} in high concentration appears to be an essential constituent of the fish tapeworm The concentration of vitamin B_{12} per gram of dried worm is from 1 33 to 3 μg easily enough to provide treatment in pernicious anemia Although the concentration of vitamin B_{12} in Ascaris lumbricoides is of the same magnitude vitamin B_{12} deficiency has not with certainty been found in cases of infestation with this parasite perhaps because of differences in quantity and biologic properties of these parasites as compared to the fish tapeworm Other parasites such as Taenia saginata contain considerably less vitamin B_{12} and do not appear capable of causing vitamin B_{12} deficiency[100]

2 Bacteria The Blind Loop Syndrome

Megaloblastic anemia may appear in association with the presence of a surgically created blind loop of intestine[51] in the presence of the multiple small blind loops of massive small in

tion The present working diagnosis is malabsorption syndrome but dietary folic acid deficiency plus a specific absorptive defect for vitamin B_1 due to lack of intestinal receptors for intrinsic factor remains an alternate possibility

The final 3 patients have been partially studied for absorptive defects other than for vitamin B_1 including three day fat balance studies with no such defects found This was also true of other cases alluded to in the literature [173, 4]

One or more of the 5 above noted cases may eventually be definitively proved to have solely a specific absorptive defect for vitamin B_1 due to a reduction or absence of intestinal receptors for intrinsic factor or of whatever other small intestinal mechanism or mechanisms which may be involved in vitamin B_1 absorption In all of these 5 cases repetitive studies at varying intervals of time are required to rule out an occult generalized malabsorption syndrome whose various other parameters may be only intermittently demonstrable Other cases of possible specific malabsorption for vitamin B_1 are being closely followed elsewhere [4]

At present it may be said that specific malabsorption for vitamin B_1 does exist but its existence as an entity independent of adult coeliac disease (and possibly other etiologic entities in the group of generalized malabsorption syndromes) cannot be considered proved until extensive longitudinal studies over a period of years have been carried out or until jejunal biopsies are obtained

It must be remembered that some patients with malabsorption syndrome may not demonstrate malabsorption for fats even on serially repeated studies and may show malabsorption only for glucose on one occasion only for vitamin A on another occasion etc

Unfortunately none of our five patients had jejunal biopsy Demonstration of a normal jejunal mucosa in these individuals would effectively exclude idiopathic steatorrhea and help to establish definitively the diagnosis of specific malabsorption for vitamin B_1

which the patients ceased to respond to parenteral vitamin B_{12} therapy and showed a return to megaloblastic hematopoiesis after developing extensive gastric carcinoma[43, 59, 60] The author has observed a possibly similar phenomenon in a 260 pound female patient with pernicious anemia in remission who developed carcinoma of the breast with extensive metastases to bones[61] She went into hematologic relapse despite continuation of her maintenance dosage of 60 μg of vitamin B_{12} parenterally at monthly intervals The patient died before it could be determined whether the hematologic picture could be improved by administration of larger amounts of vitamin B_{12} or administration of folic acid the serum vitamin B_{12} level just prior to death was 620 $\mu\mu g$/ml by L. Leishmanii assay (a normal result) but her red cell B_{12} level was only 180 $\mu\mu g$/ml (In the normal subject serum and red cell vitamin B_{12} levels are roughly the same) It is not known whether the phenomenon of relapse despite continuation of the maintenance dosage of vitamin B_{12} in these patients was a reflection of inability to utilize vitamin B_{12} increased demand for the vitamin or yet another cause such as folic acid deficiency In the light of present knowledge the latter possibility seems most likely (See section Parasitization of Folic Acid by Malignant Tissue page 59)

Recent experimental evidence in rats that vitamin B_{12} may be a co factor in amino acid incorporation into protein[62, 63] has been accompanied by demonstration of inhibition of protein biosynthesis by the anilide of the monocarboxylic acid of vitamin B_{12} which seemed to function as an anti vitamin B_{12} in this reaction[6]

Future studies using anti vitamin B_{12} agents as antimetabolites to reduce the speed of neoplastic growth in humans much as the antifolic acid agents are now used may produce clear cut cases of inability to utilize vitamin B_{12} Preliminary studies[60, 64, 65] suggest this possibility may soon be realized with the use of altered vitamin B_{12} molecules which may act by blocking the vitamin B_{12} transport and storage systems by competitive inhibition of vitamin B_{12} or both

testinal diverticulosis or with intestinal stricture or anastomosis [31-33] In such cases impaired absorption of vitamin B_{12} has been repeatedly demonstrated

The defective vitamin B_{12} absorption may be greatly improved during administration of one of the tetracycline group of antibiotics [1] but not by neomycin or sulfisoxazole [33] The response to antibiotics the avidity of certain strains of intestinal bacteria for vitamin B_{12} [3] and the evidence from autopsy studies [33] and animal experiments [4-[illegible]] that an abnormal small intestinal bacterial flora proliferating in the stagnant loops of small intestine was an invariable occurrence when macrocytic anemia developed all led to the concept that stasis of intestinal content with abnormal bacterial growth results in impaired *absorption of vitamin B_{12} and possibly of folic acid possibly by* diversion from the host to the bacteria [5,6] In some cases the major deficiency may be of folic acid rather than vitamin B_{12}

While the mechanism of this phenomenon may be direct competition for available vitamin B_{12} and folic acid in the intestine by the bacteria and the host this mechanism is not as clearly demonstrated in the blind loop syndrome as it is in fish tapeworm anemia One bit of evidence suggesting a possible role for the tetracyclines other than destruction of abnormal intestinal flora is a report of slow but definite remission of pernicious anemia on administration of chlortetracycline [7] This may have been spontaneous remission but more study of this phenomenon may be useful

In certain megaloblastic anemias in Kenya dramatic improvement has been reported after treatment with oral penicillin alone [8] It was speculated that the high carbohydrate low animal protein diet of these natives might produce an abnormal small bowel bacterial flora

III INADEQUATE UTILIZATION

A Inability to Utilize Vitamin B_{12}

The existence of this entity has not yet been established with certainty Cases of pernicious anemia have been reported in

istered dose within 24 hours (Normal persons excreted 0 to 10 per cent under similar conditions.) After giving normal plasma the urinary excretion of the same dose of labeled vitamin decreased to 24 per cent. Temporary remission could be produced by a single intramuscular dose of 90 μg of vitamin B_{12} or by the intravenous administration of 250 ml of human plasma. The patient had unexplained unresponsiveness to weekly injections of crude liver extract for 18 years prior to demonstration of her response to normal plasma administration.

In a recent personal communication, Horrigan provided further information regarding the patient, including the following:

Prior to treatment her serum vitamin B level was only 9 μμg/ml (E. gracilis assay performed by V. Lear at Thorndike Memorial Laboratory) confirming the existence of vitamin B_{12} deficiency. After three successive responses to plasma the patient became refractory to it. Her vitamin B requirement to maintain remission gradually rose to daily injections of 1,000 μg. Iron deficiency anemia developed. A bleeding *hiatus hernia was found and operatively repaired.* The patient died a month after the operation after a few days of rapidly increasing icterus and terminal bleeding from all orifices. At postmortem examination the findings were multiple endocrine tumors (chromophobe adenoma of pituitary, adenomatosis of parathyroid, islet cell tumors of pancreas, islet cell tumor of jejunum), cortical hyperplasia of adrenals, slight nephrocalcinosis, and acute massive distention of intestine.

The exact mechanisms of her defect with respect to vitamin B_{12} were never worked out. A possible relationship to an occult malabsorption syndrome or to one or more of her endocrine abnormalities was not demonstrated prior to her death. Study of other such cases, when found, may throw considerable light on the mechanism of vitamin B_{12} binding, transport, and storage.

B Parasitization by Fetus

Serum vitamin B_{12} levels gradually decrease during pregnancy and vitamin B_{12} levels of umbilical cord blood are invariably higher than those of the mother obtained at the same time [66] [69] [69a] Supraphysiologic (1000 μg) oral doses of vitamin B_{12} may be absorbed at a greater than normal rate by pregnant women [20] but depressed absorption of physiologic doses has been reported [21] This depressed absorption may be due to decreased secretion of intrinsic factor it is known that gastric secretion of acid and pepsin falls during pregnancy [3]

Despite the lowering during pregnancy serum vitamin B_{12} levels generally remain within normal limits This is not always true however [74] [7] [48b] Megaloblastic anemia rarely occurs during pregnancy when nutrition is adequate [3] [6] when it occurs with poor nutrition it is more frequently due to primary deficiency of folic acid than of vitamin B_{12} [10] [77] [81]

The combination of a marginal vitamin B_{12} intake and parasitization of available vitamin B_{12} by the fetus could lead to a vitamin B_{12} deficiency in the mother Such a deficiency has not been frequently reported but does occur [74] [7] [48b]

C Inability to Retain Absorbed Vitamin B_{12}

Most of the vitamin B_{12} in human serum is bound to serum proteins [8] primarily to an alpha 1 globulin [8] [83] It appears from studies in vitro that amounts of vitamin B_{12} in excess of physiologic serum levels may be bound to other fraction including beta globulins [84] [84]

It is possible that deficiency or absence of the vitamin B_{12} binding protein in serum could lead to inability to retain vitamin B_{12} A single case of macrocytic anemia with a defect in vitamin B_{12} binding has been reported [8] That patient s macrocytic anemia could not be maintained in abeyance by 30 μg of vitamin B_{12} intramuscularly at one to two week intervals and intramuscular administration of 0.5 μg of Co^{60} labeled vitamin B_{12} resulted in the urinary excretion of 60 per cent of the admin

is that folic acid and the chemically well defined folic acid active substances are not ordinarily present in human blood [59]

Balance studies cannot be used to study the absorption of folic acid because fecal excretion considerably exceeds dietary intake of the vitamin probably due to its synthesis by intestinal bacteria [90]

In the light of present knowledge no clear separation of the folic acid deficiency states caused by failure to ingest folic acid from those caused by failure to absorb the vitamin is possible The general heading of Nutritional Folic Acid Deficiency will therefore be used to encompass those states characterized by variable degrees of inadequate intake and inadequate absorption of folic acid The relative roles of inadequate diet and defective absorption in each condition will be indicated insofar as it is possible but the reader must realize that future knowledge may necessitate revision of the relative significance attributed to these two factors in any given clinical state

The recent demonstration that folic acid deficiency may be measured easily in man [489, 489a, 48b] may prove a stepping stone on the road to solution of many of these problems

I NUTRITIONAL FOLIC ACID DEFICIENCY

A. Inadequate Diet

The daily dietary requirement of a normal man for folic acid is estimated to be less than one mg [56, 91] The excellent hematologic response of some folic acid-deficient patients to parenteral administration of as little as 250 µg daily [84] suggests that the daily requirement may be less than 0 25 mg As stated above more studies with parenteral administration of small doses of folic acid in folic acid deficient patients are needed to delineate more clearly the minimal daily requirement for this vitamin

The major dietary sources of folic acid are animal liver and kidney and fresh green leafy (and nonleafy) vegetables [9] Dried kidney beans navy beans and soy beans are also high in folic

5 *Etiologic Classification of the Megaloblastic Anemias Due to Folic Acid Deficiency and Other Causes*

ETIOLOGIC CLASSIFICATION of the folic acid deficiency states has been hampered by a number of problems The daily dietary requirement for folic acid is unknown [56] Because of the efficacy of oral treatment of folic acid deficiency states no extensive studies have been reported indicating the minimal parenteral maintenance dose for folic acid deficient patients Therefore no baseline has been established for studies relating to the possibly different requirement for folic acid of individuals with various forms of folic acid deficiency

This is especially true of the folic acid deficiency states of pregnancy and infancy where increased requirement for folic acid almost certainly exists as a result of the increased metabolic load carried by the mother and the growth requirement of the infant

Because of the simplicity of oral treatment with 5 to 15 mg doses of folic acid in patients with intestinal malabsorption very little information is available as to whether malabsorption is the cause or the effect of folic acid deficiency in these individuals It is quite probable for example that the folic acid deficiency of many cases of tropical sprue is primarily due to an inadequate diet [10] and that the folic acid deficiency itself produces intestinal malabsorption diarrhea and perpetuation of the deficiency due to the resulting impaired absorption of folic acid

Determination of baseline blood levels of folic acid is considered valueless by most workers [7] the available evidence

is that folic acid and the chemically well defined folic acid active substances are not ordinarily present in human blood.[89]

Balance studies cannot be used to study the absorption of folic acid because fecal excretion considerably exceeds dietary intake of the vitamin, probably due to its synthesis by intestinal bacteria.[10]

In the light of present knowledge, no clear separation of the folic acid deficiency states caused by failure to ingest folic acid from those caused by failure to absorb the vitamin is possible. The general heading of "Nutritional Folic Acid Deficiency" will therefore be used to encompass those states characterized by variable degrees of inadequate intake and inadequate absorption of folic acid. The relative roles of inadequate diet and defective absorption in each condition will be indicated insofar as it is possible, but the reader must realize that future knowledge may necessitate revision of the relative significance attributed to these two factors in any given clinical state.

The recent demonstration that folic acid deficiency may be measured easily in man[489, 459, 460b] may prove a stepping stone on the road to solution of many of these problems.

I NUTRITIONAL FOLIC ACID DEFICIENCY

A. Inadequate Diet

The daily dietary requirement of a normal man for folic acid is estimated to be less than one mg.[286, 91] The excellent hematologic response of some folic acid deficient patients to parenteral administration of as little as 250 μg daily[84] suggests that the daily requirement may be less than 0.25 mg. As stated above, more studies with parenteral administration of small doses of folic acid in folic acid deficient patients are needed to delineate more clearly the minimal daily requirement for this vitamin.

The major dietary sources of folic acid are animal liver and kidney and fresh green leafy (and nonleafy) vegetables.[9] Dried kidney beans, navy beans and soy beans are also high in folic

acid content as are peanuts almonds coconuts and some fresh fruits

It should be noted that from 50 to 95 per cent of the folic acid content of foods may be lost in cooking [93] losses may be especially great in canned foods [94]

TABLES 1 AND 2 present the distribution of folic acid in foods [95] and provide a useful reference when attempting to determine whether the diet of a given patient is deficient in folic acid The values are for *fresh uncooked foods* only

It should be remembered that most diets which are deficient in folic acid are also inadequate in vitamin B_{12} though to a lesser degree A striking exception to this rule is the folic acid deficiency associated with a milk diet Milk of most kinds is uniquely low in folic acid but contains adequate quantities of vitamin B_1 [9, 96] An additional factor favoring general predominance of folic acid dietary deficiency is a possibly somewhat greater stability of vitamin B_1 and greater retention in foods with cooking

1 Megaloblastic Anemias in Infancy

Because of the low folic acid content of milk infants subsisting entirely on milk may develop megaloblastic anemia [9, 30] Concomitant ascorbic acid deficiency can often be expected especially when the infant is fed cow s milk which even when undiluted contains only one quarter as much vitamin C as breast milk [301] (See section Inadequate Utilization of Folic Acid due to Ascorbic Acid Deficiency page 58)

Evaporated milk [9] dried milk goat s milk and breast milk [9, 96] all may provide inadequate quantities of folic acid The megaloblastic anemia of infants fed goat s milk exclusively has been described as a separate entity but this anemia shows the typical recovery pattern following folic acid therapy [300]

Megaloblastic anemia following the ingestion of a diet consisting almost exclusively of milk has been reported in adults who were emotionally disturbed (usually depressed)

TABLE 1—*Folic Acid (PGA Pteroylglutamic Acid) Content of Foods*

Food	Moisture	Total PGA content fresh basis γ/g
Meats and Eggs		
Beef round steak	0	0 06-0 11
Beef heart	f	0 03
Beef kidney	76	0 48
Beef sweetbreads	78	0 93
Beef liver	68	~ 9
Lamb stew meat	49	0 03
Lamb liver	69	[illegible] 6
Pork loin	66	0 03
Pork liver	68	2 2
Pork ham (smoked)	64	0 10
Chicken dark meat	/	0 03
Chicken white meat	[illegible]	0 03
Chicken liver	2	3 7
Eggs whole	4	0 03-0 08
Egg white	87	0 001
Egg yolk	50	0 13
Nuts		
Peanuts	1	0 51
Almonds	4	0 45
Coconuts	44	0 94
Vegetables fresh		
Asparagus	93	0 89-1 17
Beans lima	0	0 10-0 40
Beans green snap	90	0 14-0 41
Beets	86	0 10-0 15
Broccoli	91	0 74-0 35
Brussels sprouts	84	0 20-0 31
Cabbage	[illegible]	0 11-0 75
Carrots	88	0 0 -0 15
Cauliflower	91	0 17-0 29
Corn sweet	64-81	0 03-0 0
Cucumbers	96	0 9 -? 07
Greens beet	90	0 31-0 39
Greens spinach	39	0 48-1 15
Lettuce	96	0 03-0 54
Peas fresh	G	0 1 -0 35
Pepper green	34	0 01-0 09
Potatoes peeled	80	0 04-0 06
Potatoes peel	73	0 08-0 0
Pumpkin	9	0 05-0 10
Sweet potato	0	0 05-0 19
Tomatoes	9 -94	0 07-0 15

From STOKSTAD E L R Pteroylglutamic acid VIII Occurrence in foods *In* Sebrell W H J and Harris R S The Vitamin New York Academic Press 19 4 vol 3

TABLE 2—*Folic Acid (PGA Pteroylglutamic Acid) Content of Foods*

Food	Moisture [illegible]	Total PGA content fresh basis γ/g
Vegetables dried		
Beans kidney	9	1 90
Beans navy	11	1 28
Beans soybeans	8	2 08
Peas green split	9	0 [illegible]4
Peas yellow split	—	0 24
Fruits fresh		
Apple whole	84	0 04
Apricots	8[illegible]	0 30
Bananas	[illegible]3	0 3[illegible]
Berries blackberries	84	0 [illegible]9–1 1
Berries cranberries	8[illegible]	0 13
Berries strawberries	91	0 60
Cantaloupes	91	0 4[illegible]–0 88
Cherries	[illegible]0	0 30
Grapefruit	[illegible]9	0 24
Grapes	80	0 [illegible]0–0 [illegible]9
Lemons	91	0 [illegible]
Oranges	86	0 [illegible]8–0 40
Orange juice	87	0 36
Peaches	88	0 08–0 20
Pears	8[illegible]	0 11–0 16
Fruits dried		
Apricots	2[illegible]	0 06
Peaches	2[illegible]	0 0[illegible]
Prunes	[illegible]	0 0[illegible]
Raisins seedless	1[illegible]	0 13
Cereals and grains		
Barley	11	0 4[illegible]
Corn yellow	11	0 [illegible]6
Oats	11	0 [illegible]
Rice brown	5	0 1[illegible]–0 3
Rice milled	[illegible]	0 15
Sorghum grain		
White kafir	11	0 [illegible]1
Yellow milo	10	0 [illegible]4
Wheat	8 [illegible]–10 [illegible]	0 34–0 4
Dairy Products		
Buttermilk	88	0 11
Evaporated	[illegible]3	0 0[illegible]
Non fat dry	[illegible]	0 0[illegible]
Cheese Cheddar	34	0 19–0 [illegible]
Cheese cottage	3[illegible]	0 [illegible]

From STOKSTAD F L R [illegible] [illegible]glutamic acid VIII Occurr[illegible]n[illegible]e in food [illegible] Sebrell W H Jr and Harr[illegible] R S The Vitami[illegible]s Ne[illegible] York [illegible] 19[illegible]4 [illegible]ol 3

and in adults in whom full mouth dental extractions led to substitution of milk for all solid food [9]

Infants with kwashiorkor or other debilitating conditions occasionally manifest a megaloblastic anemia In such cases there are so many deficiencies (often including vitamin B_{12} deficiency) that it is difficult to assign a predominant role in the development of megaloblastosis to any one of them [13] although treatment with folic acid usually is required and produces a reticulocytosis [304, 30] The one four year old boy with kwashiorkor recently seen at The Mount Sinai Hospital had a megaloblastic anemia with a serum vitamin B_{12} level of 32 $\mu\mu$g/ml a serum folic acid level of 2 7 mμg/ml and a serum iron level of 15 μg/ml The iron deficiency had a moderate masking effect on the erythrocyte component of the megaloblastic picture due to the B_{12} and folic acid deficiencies

Remission of megaloblastic anemia in infancy has occurred following vitamin B_{12} administration [306] perhaps due in the majority of cases to mobilization of residual stores of folic acid Failure of response to vitamin B_{12} is frequent [39, 307] but failures of response to folic acid therapy occasionally supplemented with ascorbic acid are relatively rare It must be concluded that in most instances megaloblastic anemia in infancy is caused primarily by deficiency of folic acid However neurologic damage has been observed frequently enough especially by European workers [307, 30] so that concomitant or even primary vitamin B_{12} deficiency must always be looked for The term megaloblastic anemia of infancy should be discarded It implies an etiologic unity which does not exist

2 Megaloblastic Anemias in Pregnancy and Puerperium

The great majority of cases of megaloblastic anemia in pregnancy has been in women ingesting diets inadequate in folic acid content [308] and often wholly inadequate especially in meat content [73] This inadequacy is accentuated by the increased requirement for folic acid during pregnancy [309] especially during the last two months when the fetal requirement for this

vitamin may be maximal[304] and overt folic acid deficiency appears

While most cases of megaloblastic anemia in pregnancy and puerperium appear to result primarily from folic acid deficiency some cases have superimposed vitamin B_1 deficiency or suffer from primary deficiency of vitamin B_1 [74, 5, 48b] Such deficiency may develop from the combination of a marginal diet depressed absorption associated with pregancy [1] with folic acid deficiency *and parasitization of the mother s vitamin B_1 by the fetus (See* section Parasitization of Vitamin B_1 by Fetus page 42)

The term megaloblastic anemia of pregnancy implies an etiologic unity which does not exist and should be discarded A more accurate designation would be megaloblastic anemia in pregnancy due to folic acid deficiency and/or vitamin B_1 deficiency caused by (state specific cause or causes such as poor diet)

Careful search for early signs of megaloblastic anemia in the peripheral blood smears of all women admitted to hospital for delivery might reveal the incidence of megaloblastic anemia in pregnancy to be as high as 1 in every 39 patients in temperate zones[2, 3] or even higher The incidence in the tropics is still higher

Some cases have been reported in the presence of a presumably excellent diet and no known gastrointestinal disturbance[310] However such cases responded to folic acid therapy thus leaving open the question as to etiology

It is undecided as to whether impaired absorption of folic acid occurs during pregnancy Girdwood[311] found no evidence for inadequate absorption but more recent work[31] indicates that absorption of folic acid may be impaired in pregnant women[304, 312]

The onset of megaloblastic anemia in pregnancy usually occurs in the last trimester and sometimes in the puerperium[313, 313] Remission may appear spontaneously when the pregnancy *terminates but relapse may occur* with a subsequent pregnancy or in the absence of another pregnancy [76]

In the tropics megaloblastic anemia of pregnancy may respond partially or completely to vitamin B_{12} alone [7, 313-317] In temperate zones this may occur[317] but is much less common probably because general dietary deficiency stemming from economic and religious reasons with inadequacies of folic acid vitamin B_{12} and protein is much *more* common in tropical countries[318]

Large doses of vitamin B_{12} often produce hematologic improvement[319, 3 0] This may be due to a mass action effect of such doses with mobilization of residual stores of folic acid Such an effect is suggested by the fact that while vitamin B_{12} may induce a temporary remission the end result of such therapy is disappointing in many cases if it is not supplemented with folic acid[3 1] In some cases of course primary vitamin B_{12} deficiency may exist

3 Nutritional Megaloblastic Anemia (Tropical and Nontropical Macrocytic Anemia)

Gross dietary deficiency resulting from poverty ignorance or religious tenets is widespread throughout the world and especially in tropical areas Such deficiency often encompasses a lack of animal protein and of fresh green vegetables[6, 3] It therefore results in variable degrees of folic acid and vitamin B_{12} deficiency in addition to deficiency of iron and sometimes of protein as well[7]

Many patients with nutritional megaloblastic anemia respond partially or completely to vitamin B_{12} [11, 314, 316, 3 3, 3 4] Slow and suboptimal responses are frequent even with large doses of vitamin B_{12}

On the other hand a uniformly excellent response follows folic acid administration[6, 11, 3, 3 8]

Subnormal serum vitamin B_{12} levels have been reported in some cases of nutritional megaloblastic anemia[10, 3 9, 3 0] but even in such cases folic acid was usually required in addition to vitamin B_{12} in order to obtain complete response to treatment

Therefore the megaloblastic anemia resulting from inade

vitamin may be maximal[304] and overt folic acid deficiency appears.

While most cases of megaloblastic anemia in pregnancy and puerperium appear to result primarily from folic acid deficiency, some cases have superimposed vitamin B_1 deficiency or suffer from primary deficiency of vitamin B_1.[4, 7, 489b] Such deficiency may develop from the combination of a marginal diet, depressed absorption associated with pregancy,[4] with folic acid deficiency and parasitization of the mother's vitamin B_1 by the fetus. (See section "Parasitization of Vitamin B_1 by Fetus," page 42.)

The term "megaloblastic anemia of pregnancy" implies an etiologic unity which does not exist and should be discarded. A more accurate designation would be "megaloblastic anemia in pregnancy due to folic acid deficiency and/or vitamin B_1 deficiency caused by (state specific cause or causes, such as poor diet)."

Careful search for early signs of megaloblastic anemia in the peripheral blood smears of all women admitted to hospital for delivery might reveal the incidence of megaloblastic anemia in pregnancy to be as high as 1 in every 39 patients in temperate zones[73] or even higher. The incidence in the tropics is still higher.

Some cases have been reported in the presence of a presumably excellent diet and no known gastrointestinal disturbance.[310] However, such cases responded to folic acid therapy, thus leaving open the question as to etiology.

It is undecided as to whether impaired absorption of folic acid occurs during pregnancy. Girdwood[311] found no evidence for inadequate absorption, but more recent work[31] indicates that absorption of folic acid may be impaired in pregnant women.[304, 312]

The onset of megaloblastic anemia in pregnancy usually occurs in the last trimester, and sometimes in the puerperium.[313, 313] Remission may appear spontaneously when the pregnancy terminates, but relapse may occur with a subsequent pregnancy or in the absence of another pregnancy.[70]

usually reveals a definite shift to the right as expected in early megaloblastic anemia

B Defective Absorption

As was stated in the discussion of the vitamin B_1 deficiency *states associated with small intestinal disorders (vide supra)* variable degrees of reduced absorption of folic acid and of vitamin B_1 may occur with any structural or functional disorder of the small intestine If the disorder is chronic clinically overt deficiency may eventually occur Similar deficiency may occur in the presence of an abnormal intestinal flora usually resulting from stagnation of intestinal contents (the blind loop syndrome) It is possible to infer that folic acid is absorbed primarily from the jejunum Patients who have had large amounts of ileum resected may retain normal serum folic acid levels even a decade after the resection[48ab] Patients with idiopathic steatorrhea have lost much of their jejunal absorptive surface but still may have normal ileal mucosa[3, 0] Such patients invariably have low serum folic acid levels and the levels may not rise to normal even after prolonged therapy with steroids or gluten free diets[48(b), 1]

1 Malabsorption Syndrome

a *Primary*

(1) *Tropical sprue* Nearly all cases of tropical sprue appear to be closely related to deficiency of folic acid Treatment with folic acid not only converts the hematologic picture to normal but also brings about subsidence of the anorexia diarrhea steatorrhea and other clinical signs and symptoms of tropical sprue[331, 33]

It is probable that the malabsorption syndrome of tropical sprue with its accompanying defective absorption of folic acid[31, 336] is the end result of dietary deficiency of folic acid and should be classed as a *pseudo primary* malabsorption syndrome resulting from dietary deficiency of folic acid Such die

quate diets may be considered in almost all cases to be primarily a consequence of folic acid deficiency In the production of these megaloblastic anemias it is not yet determined whether the major role is played by a relatively greater deficiency of folic acid than of vitamin B_{12} in the diet or relatively greater body stores and slower utilization of vitamin B_{12} The author favors the latter possibility in the New York area because most of the patients with nutritional megaloblastic anemia of which he has personal knowledge were ingesting diets grossly inadequate in both vitamin B_{12} and folic acid and yet they had normal serum vitamin B_{12} levels despite low serum folic acid levels

4 Cirrhosis Associated with Chronic Alcoholism

Megaloblastic bone marrow pictures were observed in 4 of a group of 16 patients with macrocytic anemia associated with hepatic cirrhosis and chronic alcoholism[84] In the patients with megaloblastic anemia demonstrable histologically folic acid deficiency was proved by observation of subnormal daily urinary excretion of folic acid failure to excrete administered folic acid promptly and striking clinical and hematologic response to oral administration of 1350 μg of conjugated folic acid plus 150 μg of free folic acid daily

The evidence suggested that folic acid deficiency developed in these patients through a combination of marginal or inadequate dietary intake plus a possible increased requirement for folic acid While no evidence of folic acid deficiency was found in the 12 patients with macrocytic anemia *without* megaloblastic marrows the possibility of incipient folic acid deficiency in those cases was not completely ruled out

Further exploration of the etiology of the macrocytic anemias so often associated with hepatic disease is needed[13] Our own experience *with patients with cirrhosis and macrocytic anemia* would suggest that folic acid deficiency is usually present manifested by low serum folic acid levels regardless of whether the bone marrow morphology is clearly megaloblastic or appears to be normoblastic In these latter patients an Arneth count

usually reveals a definite shift to the right as expected in early megaloblastic anemia

B Defective Absorption

As was stated in the discussion of the vitamin B_1 deficiency states associated with small intestinal disorders (*vide supra*) variable degrees of reduced absorption of folic acid and of vitamin B_1 may occur with any structural or functional disorder of the small intestine If the disorder is chronic clinically overt deficiency may eventually occur Similar deficiency may occur in the presence of an abnormal intestinal flora usually resulting from stagnation of intestinal contents (the blind loop syndrome) It is possible to infer that folic acid is absorbed primarily from the jejunum Patients who have had large amounts of ileum resected may retain normal serum folic acid levels even a decade after the resection [489b] Patients with idiopathic steatorrhea have lost much of their jejunal absorptive surface but still may have normal ileal mucosa [330] Such patients invariably have low serum folic acid levels and the levels may not rise to normal even after prolonged therapy with steroids or gluten free diets [489f, 12]

1 Malabsorption Syndrome

a *Primary*

(1) *Tropical sprue* Nearly all cases of tropical sprue appear to be closely related to deficiency of folic acid Treatment with folic acid not only converts the hematologic picture to normal but also brings about subsidence of the anorexia diarrhea steatorrhea and other clinical signs and symptoms of tropical sprue [331, 335]

It is probable that the malabsorption syndrome of tropical sprue with its accompanying defective absorption of folic acid [31, 336] is the end result of dietary deficiency of folic acid and should be classed as a *pseudo primary* malabsorption syndrome resulting from dietary deficiency of folic acid Such die

tary deficiency has been observed to be a significant factor in the pathogenesis of the disorder [10–1]

Clear delineation of the pathogenesis has been confused by the demonstration of impaired absorption of folic acid in tropical sprue[31 38] and of return to normal folic acid absorption following the administration of a course of antibiotics[31] The latter observation suggests the possibility that changes in the bacterial flora of the intestine perhaps related to diet [8] may be intimately involved in the development of the folic acid deficiency state

Delineation of the etiology of tropical sprue has been further confused by including in this category individuals with non tropical sprue who happened to be in the tropics [18] Unlike in nontropical sprue the associated atrophy of the intestinal villi in tropical sprue often disappears on treatment [23] and complete recovery of the patient is very frequent [16 1 331 4]

(2) *Childhood and adult coeliac disease (idiopathic steatorrhea nontropical sprue)* Unlike tropical sprue the existence of which as a clear cut condition independent of dietary folic acid deficiency is in doubt the existence of childhood and adult coeliac disease as a single etiologic entity is becoming firmly established [04 6 8 33] Its existence as a hereditary constitutional defect in metabolism associated with irreversible atrophy of the small intestinal villi has been discussed in connection with the development in this condition of a secondary absorptive defect for vitamin B_1

It is probable that the metabolic defect is enzymatic and associated with folic acid metabolism [04] but it is undetermined whether the primary defect is in the transport of folic acid or its conjugates across the intestinal barrier or in their utilization after absorption Poor transport across the intestinal mucosa is suggested by the low fasting serum folic acid levels of patients with idiopathic steatorrhea before *and after* years of treatment with steroids or a gluten free diet

In this connection determination of the fasting serum folic acid level in the relatives of patients with idiopathic steatorrhea

may prove fruitful. Such a study might uncover a number of persons with latent disease manifested only by a serum folic acid level slightly below normal and thereby provide information of value in further delineating the genetic pattern of idiopathic steatorrhea.

The anemia of childhood coeliac disease is usually microcytic and hypochromic, while that in the adult expression of the disorder is macrocytic.[28, 337, 338] A rare case of macrocytic anemia may be found in childhood coeliac disease.[339]

The megaloblastic anemia which occurs in the adult form of coeliac disease generally responds to folic acid administration[340] and incompletely or not at all to vitamin B_{12}.[341, 343] Unlike in tropical sprue, however, in nontropical sprue treatment with folic acid is not usually associated with a general improvement of symptoms and signs other than those due to the megaloblastic anemia, and not infrequently some anemia and macrocytosis may persist.[344–348] When this occurs, associated vitamin B_{12} deficiency may be found.

b. *Secondary*

Folic acid deficiency is rare in those conditions producing a secondary malabsorption syndrome without the growth of an abnormal intestinal bacterial flora (such as may accompany intestinal strictures and anastomoses). The latter conditions will be discussed below.

C. Competition for Folic Acid by Intestinal Bacteria: The Blind Loop Syndrome

As stated in the discussion of this syndrome under the heading of the vitamin B_{12} deficiency states, the major deficiency in any given case of blind loop syndrome may be of either folic acid or vitamin B_{12}.

Cases have been described in which the associated megaloblastic anemia failed to respond to vitamin B_{12} but was adequately treated with folic acid.[6] The reverse situation has also been documented.[33]

It may be that the type of deficiency which predominates will depend on the relative requirement and avidity for folic acid and vitamin B_1 of the abnormal bacterial intestinal flora associated with the particular case of blind loop syndrome

II INADEQUATE UTILIZATION OF FOLIC ACID

A Inability to Utilize

1 Due to Administration of Folic Acid Antagonists

Agents such as 4 aminopteroyl glutamic acid (Aminopterin) by blocking the utilization of folic acid can produce a megaloblastic anemia [340, 384]

This anemia is not reversible by folic acid administration since 4 amino analogs of folic acid not only compete with folinic acid but also block the conversion of folic acid to folinic acid [3 0] It may be reversed by administration of citrovorum factor (folinic acid) which bypasses the block [3 0 3 1]

2 Due to Administration of Anticonvulsants or Pyrimethamine

The appearance of megaloblastic anemia in patients being treated for prolonged periods of time with anticonvulsant drugs has been reported with increasing frequency [35, 3 3] Drugs involved include Dilantin (Phenytoin)(diphenylhydantoin sodium) [354, 357] Primidone (Mysoline)(5 phenyl 5 ethyl hexahydropyrimidine 4 6 dione) [3 8, 360] Rutonal (methophenobarbital) [361] and occasionally other barbiturates including Amytal (amylobarbitone) and Seconal (quinalbarbitone) [3 3, 36] Megaloblastic anemia has not yet been reported following the use of phenobarbital probably because of the low dose of this agent usually administered [61]

The megaloblastic anemia which occurs responds either to withdrawal of the anticonvulsant [363] or to administration of folic acid [3 4 3 9] and weakly or not at all to vitamin B_1 except in very large doses [3, 3 3, 364]

The absorption and tissue stores of folic acid in these patients appear to be normal in some but in others the clearance of intravenously injected folic acid was abnormally rapid [304, 365] sug

gesting a possible deficiency in tissue stores of the vitamin Other interpretations of this finding are possible and it is believed at present that the anticonvulsants must in some manner interfere in the utilization of folic acid It is possible they act in part by competing with folic acid for intestinal absorption

The chemical structure of the anticonvulsants resembles the *pteridine ring system of folic acid with the pyrazine ring open* and partly degraded They also resemble therefore the pyrimidines involved in nucleoprotein synthesis and it is possible they may interfere with pyrimidine utilization as well as folic acid utilization and may produce a megaloblastic anemia in part by blocking pyrimidine incorporation into nucleoproteins

The same may be true of pyrimethamine (Daraprim) a substituted pyrimidine (2 4 diamino 5 p-chlorphenyl 6 ethylpyrimidine)[366] used as an antimalarial drug In large doses this agent *can produce a megaloblastic anemia which rapidly clears when* the drug is withdrawn[367 368] The antimicrobial action of pyrimethamine for Streptococcus faecalis is blocked by either large amounts of folic acid or small amounts of folinic acid[369] and its toxic effect on the growing rat is alleviated by folinic acid but not by folic acid[3 0] Thus while structurally pyrimethamine is more closely related to thymine than to folic acid existing evidence suggests it is more an antifolic acid than an antithymine[371]

Serum vitamin B_1 levels have usually been reported to be normal *in cases of megaloblastic anemia due to anticonvulsant* therapy[353] but low levels were found in 2 cases[2 3 3 2] In one of these cases *the low serum level of vitamin B_1 was observed* during folic acid therapy[3 3] Generally vitamin B_1 deficiency is probably not a phenomenon of significance in the megaloblastic anemias resulting from anticonvulsant therapy

A 63 year old man who had been taking Dilantin for 15 years was seen at the Mount Sinai Hospital with a megaloblastic anemia His serum folic acid level was normal but his serum vitamin B_1 level was 32 $\mu\mu g$ ml (E Gracilis assay) *In vitro* assay of his gastric juice revealed no intrinsic factor and Glass and Schilling *testing revealed inability to absorb vitamin B_{12}* cor

rectible with orally administered intrinsic factor concentrate The patient was thus revealed as a person with pernicious anemia The Dilantin was a red herring in his case revealing the necessity of careful work up for other possibilities even when a seemingly obvious explanation for megaloblastic anemia presented itself

3 Due to Ascorbic Acid Deficiency

There is evidence that ascorbic acid is involved in augmenting the conversion of folic acid to a metabolically more active form[373, 374] A few cases of megaloblastic anemia appearing in patients with scurvy and cleared by treatment with ascorbic acid have been reported[37, 377]

However the anemia associated with scurvy is usually normocytic[378] and even in severe scurvy anemia may not occur at all[3 3 0 3 8 3 9] For these reasons plus the fact that it has not been possible to produce anemia in man by experimental ascorbic acid deprivation[380, 381] it is probable that megaloblastic anemia appears in ascorbic acid deficiency states only in the presence of an associated deficiency in the supply or metabolism of folic acid[37]

Dietary deficiency of both ascorbic and folic acids has been implicated in the causation of the megaloblastic anemias of infancy[38, 90, 38, 383] (See section Megaloblastic Anemias in Infancy page 46)

B Parasitization

1 By Fetus

It was pointed out in the discussion of megaloblastic anemia in pregnancy that the majority of patients were ingesting a diet inadequate in folic acid content The folic acid requirement of the rapidly growing fetus probably results in removal of a significant portion of the marginal amount of folic acid absorbed[309] and overt folic acid deficiency with megaloblastic anemia is the result The serum folic acid levels of newborns are almost in

variably much higher than the levels in their mother's serum[69a]

The low serum folic acid levels of women in the latter half of pregnancy after an oral dose of folic acid have been interpreted as due to impaired absorption of the vitamin[31] An alternative explanation may be rapid uptake by the fetus with the result that measurable serum levels in the mother are low

2 By Malignant Tissue? (See Chapter 5 Other Causes)

A megaloblastic bone marrow picture has been observed in association with various malignant diseases[384 387] It may be particularly prominent in erythremic myelosis (DiGuglielmo's disease)[388 390] and erythroleukemia (the DiGuglielmo syndrome)[2 388 390] and in acute leukemia usually of the aleukemic type presenting as a refractory macrocytic anemia[47 2 9 394 391 396] It is also not uncommon in polycythemia vera[397]

An explanation for the megaloblastic marrow picture associated with widespread malignant disease was speculatively derived from the observations that in such disease there is a markedly reduced output of a test dose of folic acid[351] and rapid clearance of an intravenous dose of this vitamin[304] It was suggested that the reduced output in the urine was a result of utilization of folic acid at a rapid rate by rapidly growing neoplastic tissue Such utilization could produce depletion of the body stores of folic acid or perhaps just local depletion at the bone marrow level especially if the diet contained only a marginal quantity of the vitamin and megaloblastic anemia would then appear Serum folic acid is sometimes low with widespread malignant disease[186b]

It is also possible that the marrow cells may be unable to capture or utilize folic acid or vitamin B_{12} adequately in the presence of some malignancies[396]

III ACHRESTIC ANEMIA (IDIOPATHIC REFRACTORY MEGALOBLASTIC ANEMIA)

Achrestic anemia and idiopathic refractory megaloblastic anemia are terms coined to classify those cases of megaloblastic

anemia which did not seem to fit into any other category or to respond to liver therapy [398, 399]

In some of these cases megaloblastosis disappears when folic acid therapy is administered leaving a hyperplastic bone marrow and a refractory normoblastic anemia The megaloblastosis appeared to be a consequence of excessive utilization of folic acid by the hyperplastic bone marrow [400]

The majority of these cases have responded to folic acid or liver extract and it is probable that with the currently available clinical and laboratory procedures most of these cases insofar as megaloblastic picture is concerned will be classified among the folic acid deficiency states [39] A few may be among the vitamin B_{12} deficiency conditions and the very few which do not *respond to either vitamin B_{12} or folic acid* [401, 401, 401b, 415] *may* be due to other possible causes of interference with nucleoprotein synthesis such as those discussed in the next section

OTHER CAUSES

In CHAPTER 3 The Biochemical Basis of Megaloblastic Hematopoiesis it was pointed out that the megaloblast is the product of interference with nucleoprotein metabolism This interference may be caused either by direct inhibition or by depletion of the enzyme systems and building blocks necessary for nucleoprotein synthesis

Although depletion of vitamin B_{12} or of folic acid is the cause of the overwhelming majority of megaloblastic anemias such depletion has not as yet been generally considered to be present in some conditions which are occasionally accompanied by megaloblastic bone marrow pictures

These conditions may be broadly characterized as associated with inhibition or depletion of the bone marrow [2] They include agranulocytosis [40, 403] myelofibrosis [404] tuberculosis [405, 40] leishmaniasis [406] sepsis [407] hemochromatosis [408, 409] radiation injury [407] and various severe anemias especially when associated with hemolysis [410–41, 413]

Future study may relate many of the megaloblastic cell

pictures associated with depletion of the bone marrow to deficiency of folic acid or vitamin B_{12} in some cases with such deficiency only manifest at the bone marrow level caused by inadequate or rapid utilization of these vitamins Excessively rapid clearance of an intravenously administered dose of folic acid has been demonstrated in 5 cases of chronic myelofibrosis 1 of which had megaloblastic erythropoiesis which became normoblastic after oral folic acid therapy [304] Rapid clearance of intravenously administered folic acid also occurs in hemolytic anemia [304] Even in the absence of an overtly megaloblastic bone marrow in hemolytic anemia folic acid in pharmacologic doses may produce a reticulocytosis [413]

The logical belief that megaloblastic anemia in association with various hemolytic anemias may be the result of an increased utilization of folic acid in the presence of accelerated hematopoiesis is becoming increasingly popular [413]

Megaloblastic anemia associated with hemochromatosis has been demonstrated to respond to vitamin B_{12} and folic acid administration [409, 414] There is evidence that some cases may result from a primary deficiency of vitamin B_6 [41, 416] but in only 1 case so far has this relationship been convincingly reported

Direct interference with nucleoprotein synthesis may play a role in some cases of megaloblastic anemia associated with what appears to be inhibition of the bone marrow such as agranulocytosis Here too however the possibility of folic acid deficiency must not be overlooked

Aplastic anemia is frequently associated with macrocytosis sometimes partly explainable by reticulocytosis since reticulocytes are large red cells [417] Some cases of aplastic anemia and of aregenerative anemia limited to the red cell series may be due to total bone marrow exhaustion as the end result of prolonged deprivation of vitamin B_{12} (or folic acid) which may have passed through a megaloblastic stage before eventuating in a hypocellular nonmegaloblastic bone marrow [418, 418a] Such a marrow may be capable of only a slow response if any to long term administration of vitamin B_{12} and folic acid Widespread aplasia

of the bone marrow as a terminal event was a common finding at autopsy of patients with pernicious anemia in the years prior to the discovery of liver therapy [419]

A rare patient with megaloblastic anemia may have an esoteric defect along the synthetic pathway leading to nucleoprotein formation One such patient was an infant with a refractory megaloblastic anemia associated with orotic acid excretion [401b] The anemia responded only to steroids and a concentrated yeast extract containing a mixture of uridylic and cytidylic acids The infant was believed to be unable to synthesize pyrimidine nucleotides adequately due to a block at the stage of conversion of orotic acid to orotidylic acid It is unknown whether the infant might have been able to effect this conversion with the aid of folinic acid or of one of the B_1 co enzymes which have been suggested [411] possibly to be the main forms in which vitamin B_1 is catalytically active in the enzymatic reactions of living cells It has been suggested that conditions may exist in which because of a block in the conversion of vitamin to co enzyme only the latter may have therapeutic value [419]

The subject of possible enzymatic defects in nucleoprotein synthesis in man related or unrelated to defects in conversion of folic acid folinic acid or vitamin B_1 to catalytically active forms is just beginning to be explored Careful study of all patients with refractory megaloblastic anemia with the aid of a biochemical orientation will undoubtedly uncover many exotic defects in the future

The megaloblastic anemia picture sometimes seen in association with various widespread malignant diseases has been discussed in the section Parasitization of Folic Acid by Malignant Tissue Future studies may well require reclassification of some or all of the megaloblastic anemias and megaloblastic bone marrow pictures associated with malignant disease into entirely different etiologic categories At present knowledge is too limited to permit definitive classification

6 Clinical Picture

A CENTURY AGO Addison described the clinical picture in pernicious anemia.[98] This description, which we now know may fit equally well an advanced case of any of the vitamin B_{12} deficiency states and in most respects fits the folic acid deficiency states as well, has not been improved upon. Addison wrote in 1855:

The countenance gets pale, the whites of the eyes become pearly, *the general frame flabby rather than wasted*; the pulse perhaps large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement. There is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness on attempting it; the heart is readily made to palpitate; the whole surface presents a blanched, smooth and waxy appearance; the lips, gums and tongue seem bloodless; *the flabbiness of the solids increases*; the appetite fails; extreme languor and faintness supervene, breathlessness and palpitation being produced by the most trifling exertion or emotion; some slight oedema is probably perceived about the ankles. The debility becomes extreme; the patient can no longer arise from his bed; *the mind occasionally wanders*; he falls into a prostrate and half torpid state, and at length expires. *Nevertheless, to the very last, and after a sickness of perhaps several months' duration, the bulkiness of the general frame and the obesity often present a most striking contrast* to the failure and exhaustion observable in every other respect.

The only parts of Addison's classic description which do not always fit the folic acid deficiency states as generally as those due to vitamin B_{12} deficiency have been italicized. While nervous instability may occur in folic acid deficiency, cerebral or other neurologic manifestations as a direct result of the deficiency have only been documented with lack of vitamin B_{12}.[33] The neurologic signs and symptoms which may be seen frequently in the malabsorption syndrome are probably not due to the folic acid deficiency but rather to vitamin B_{12} deficiency.[40] Subnormal serum vitamin B_{12} levels (usually below 50 μg/ml.) were observed in every case of malabsorption syndrome with neurologic signs or symptoms seen by the author.

The other feature of Addison's description which is peculiar to vitamin B_{12} deficiency is persistence of bulkiness of the frame and obesity. In the folic acid deficiency states, perhaps because

they are so often accompanied by many other deficiencies emaciation is more usual [4 1]

It is generally believed that nervous system involvement only occurs with vitamin B_{12} deficiency and not with folic acid deficiency alone [33 4 0 4] It has been suggested that this may be due to a requirement of the nervous system for vitamin B_{12} but not for folic acid [4 3] Intensive protein synthesis is required to maintain the integrity of axons [4 4 4 6] Protein synthesis is dependent on adequate production of RNA [4 7] which in turn is influenced considerably by vitamin B_{12} [4 4 6 4 8 4 9] and perhaps slightly less by folic acid [430]

Folic acid appears to be involved intimately in DNA synthesis [430 431] and only indirectly in protein synthesis These findings plus recent evidence suggesting the possibility that vitamin B_{12} may be involved in the enzymatic incorporation of amino acids into proteins [4 43 433] may help explain why nervous system damage occurs only in the presence of vitamin B_{12} deficiency and not in the presence of folic acid deficiency alone However it should be noted that the direct involvement of vitamin B_{12} in protein synthesis has been questioned [434 43] and the mechanism whereby only vitamin B_{12} but not folic acid deficiency leads to nervous system damage remains a subject for much more study

Another subject for future study is the possibility that vitamin B_{12} deficiency may result in inadequate synthesis of the lipoid moiety of the lipoproteins which are normally present in high concentrations in erythrocyte envelopes and the myelin sheaths in both the central and peripheral nervous system [4 4 6] Inadequate maintenance of the myelin sheaths due to vitamin B_{12} deficiency but not folic acid deficiency is an attractive possible explanation for the neurologic damage associated with the former condition but not the latter

The classic association of subacute combined degeneration (combined system disease posterolateral sclerosis funicular degeneration) with vitamin B_{12} deficiency is well known [437 438] It should be noted however that the disease usually starts insidiously and not subacutely combined lesions are often absent

and lesions of the peripheral nerves occur much more frequently and much earlier than lesions of the central nervous system [4, 6, 439, 441]

The use of the term subacute combined degeneration to include all of those changes in the nervous system which may occur as a result of vitamin B_1 deficiency should probably be discontinued [4, 6, 44] The nervous system changes would be more accurately described by direct reference to the actual involvement (i e peripheral nerve spinal cord or cerebral damage due to vitamin B_{12} deficiency [33, 44]) or in general terms vitamin B_1 neuropathy [4, 6]

Paresthesia especially numbness and tingling in the hands and feet is the most frequent neurologic symptom of vitamin B_1 deficiency[33, 438, 443] Not uncommonly other signs are present diminution of vibration and position sense unsteadiness poor muscular coordination with ataxia moodiness mental slowness poor memory confusion agitation and depression delusions hallucinations psychosis (usually with paranoid ideation) and central scotomata sometimes with dim vision due to optic atrophy or tobacco amblyopia[6, 33, 439, 444, 446, 446] The wide variety of sensory and motor changes tend to be symmetric especially if maintained for a period of weeks or months [447] This is not invariably so however

In the author s experience loss of position sense in the index *toes and loss of vibration sense as tested by a 256 vibrations* per second tuning fork usually precedes by many months any evidence of loss of position sense in the great toes or of vibration sense with a 128 vibrations per second tuning fork Recently a patient with addisonian pernicious anemia in relapse was seen who had just been given a clean bill of health by an excellent neurologist Examination with a 256 tuning fork revealed complete loss of vibration sense from toes to iliac crests bilaterally although the neurologist s finding of no loss of vibration sense (using a 128 tuning fork) was confirmed Also while the neurologist s finding of no loss of position sense (in the great toes) was confirmed position sense was absent in both

index toes The author has not been able to find in the literature the incidence of loss of 256 vibration sense and index toe position sense in a random sampling of the population and so cannot state with certainty that these findings by themselves are of great significance They do seem to have a definite relation to early vitamin B_1 deficiency disease and when present with such deficiency usually disappear after treatment with vitamin B_1

It is extremely important to realize that nervous system involvement may occur in the absence of overt anemia Such involvement may be present for years and irreversible damage may occur if the proper diagnosis is made too late[33, 444, 449]

In the author s experience with cases of vitamin B_1 deficiency disease with neurologic involvement in the absence of overt anemia (and of folic acid administration) careful examination of the peripheral blood always revealed the presence of an increase over the normal number of neutrophilic polymorpho nuclear leukocytes with hypersegmented nuclei and also some macroovalocytosis (see CHAPTER 3) Examination of the bone marrow revealed changes suggesting early megaloblastic hemato poiesis large erythrocyte precursors with less than the usual amount of clumping of the nuclear chromatin especially in the early stages of development (i e intermediate megaloblasts) and large but perhaps not quite giant metamyelocytes

Information as to the existence of similar hematologic clues in the reported cases of vitamin B_{12} deficiency with nervous system involvement and no overt anemia is sparse It is quite possible that similar findings were present but not commented upon as was the case in one report[449]

The importance of these hematologic clues is best illustrated by case reports

1 McL a 63 year old white man of Irish parentage was first seen at the Montefiore Hospital in May 19 8 when he was admitted to the emp r vate service for evaluation of a recent progression of Parkinson s disease which had been only slowly progressive during the past five years He lived alone ate a poor diet drank a quart of beer daily and had at one time been an alcoholic For three years he

had manifested steadily progressive mental confusion and forgetfulness On several occasions he had wandered out into the street and not known where he was At times he had gone to work on holidays when his place of work was closed His judgment was noted by relatives to be progressively poorer

On admission he was obese and plethoric and mildly confused and disoriented Slight hepatomegaly was noted Routine examination of the peripheral blood revealed a hematocrit of 41.5 per cent hemoglobin 13.8 Gm red cell count (obtained after examination of the peripheral blood smear suggested the proper diagnosis) *3 1 million white cell count 4 00 with 58 per cent neutrophilic* polymorphonuclear leukocytes 24 per cent lymphocytes 8 per cent monocyte and 10 per cent eosinophils Marked macroovalocytes and a slight increase in the number of neutrophilic polymorphonuclear leukocytes with hypersegmented nuclei were noted

Because of the peripheral blood findings a sternal bone marrow aspirate was obtained Some large but no giant metamyelocytes were seen some of the erythroid precursors were large with nuclear chromatin more finely divided than that normally seen Not one of the 10 hematologists to whom the specimen was submitted for examination believed that the marrow could be classified as *megaloblastic Indeed one hematologist of wide experience who examined the* specimens after being asked to look at them for evidences of vitamin B deficiency stated There is a mild erythroid hyperplasia without maturation arrest this specimen is not consistent with vitamin B_{12} deficiency

Nonetheless serum was obtained for assay of vitamin B_{12} content and a urinary excretion test of a modified Schilling type was started[40, 45] Prior to and shortly after the start of this test the patient dressed and attempted to leave the hospital without notifying anyone *On the first occasion he was brought back to his ward* by a police officer who found him wandering aimlessly on the street The patient claimed he left because there were thirty people after him A psychiatrist called in consultation made the diagnosis of an organic psychosis associated with cerebral arteriosclerosis

Because of the necessity for constant supervision and despite the fact that a definite lessening of his confusion was noticeable within two days after the first 1 mg injection of vitamin B_{12} given in the course of the urinary vitamin B excretion test it was considered necessary that he be transferred to a mental institution Transfer was arranged to the closed psychiatric ward of the Bronx Municipal Hospital Center

At this time the study results were obtained The serum B level was 96 μμg /ml by Euglena gracilis assay[4] This low result was coupled with the result of the urinary excretion test and the diagnosis of pernicious anemia was made (The urinary excretion test demonstrated that the patient lacked intrinsic factor since he excreted only 0 /6 per cent of the orally administered dose of labeled vitamin B_{12} when it was given alone but 10.2 per cent of the dose when it was *given with a standard amount of hog intrinsic factor concentrate*)

These results were communicated to the staff of the closed psychiatric ward where the patient resided and treatment was instituted with 1 mg of vitamin B_{12} daily After three weeks of such treatment his psychiatric *status had*

improved to the point that it was feasible to discharge him with the diagnosis of incipient addisonian pernicious anemia with cerebral vitamin B[illegible] deficiency improved by treatment

Had the significance of the macroovalocytes and hypersegmented polymorphonuclear leukocytes in the peripheral blood of this patient been overlooked he would have probably spent the rest of his life in a mental institution as just another case of senile psychosis

Part of this patient s mental disturbance may have been related to factors other than vitamin B_1 deficiency since he had been a chronic alcoholic and since the possibility of some cerebral arteriosclerosis did exist It is always difficult to be certain that a psychosis or other neurologic system damage derived purely from vitamin B_1 deficiency The major criterion is response to vitamin B_1 therapy Unless such response is complete it may be impossible to determine how much of the failure to return to complete normality is due to irreversible damage related to the length of time the patient was deficient in vitamin B_1 and how much is due to other causes This problem frequently arises in the many patients with both pernicious anemia and diabetes mellitus either of which is capable of producing severe peripheral neuropathy

Another case in which vitamin B_1 deficiency appeared almost certainly to be the only agent involved in the psychosis now follows

The patient was a 39 year old man who was seen by a psychiatrist in March 19[illegible] because of slight ataxia and paranoid ideation Diminished position and vibration sense in the lower extremities were noted but not further evaluated in terms of possible vitamin B[illegible] deficiency The patient became increasingly paranoid and in September 19[illegible] was admitted to a private sanitarium for treatment of paranoid schizophrenia which was clearly evident even to the casual observer One day in December 19[illegible] after he had received 1[illegible] electroshock treatments *with mild benefit it was noted that he was pale* Hematologic workup revealed a megaloblastic anemia with a hemoglobin of 5 8 Gm 100 ml of blood He was started on treatment with vitamin B[illegible] and transferred to the Bronx Municipal Hospital Center from which institution he was discharged in seven weeks no longer psychotic [illegible] with a [illegible] diagnosis of [illegible] pernicious anemia to return to his wife and children

Had he not been lucky enough to develop an anemia severe enough to be recognized with the unaided eye this man might have gone on to develop irreversible cerebral damage as well as severe combined system disease

These cases and many others seen by the author and many prior workers emphasize the importance of screening psychotic patients for vitamin B_{12} deficiency by careful examination of the peripheral blood smear determination of the serum vitamin B_{12} level or both[33, 416] Such a screening procedure carried out at large mental institutions might prove of sufficient value to pay for itself many times over in saved patient days Similar screening might prove fruitful in patients whose mental changes are not serious enough to require hospitalization but in whom senility is considered to explain adequately their behavior

Another case demonstrating the importance of careful examination of the peripheral blood smear as a routine procedure while perhaps not as dramatic as the first case now follows

M I a 45 year old lady was admitted to the semiprivate service of the Montefiore Hospital in April 19[illegible]8 for evaluation because of an attack of syncope Similar episodes of syncope had occurred intermittently for the three years prior to admission and there had been a single syncopal episode 20 years previously

Routine hematologic workup revealed a hematocrit of 33.5 per cent hemoglobin 10.4 Gm red count 3.35 million and white count 4400 with 60 per cent neutrophilic polymorphonuclear leukocytes 32 per cent lymphocytes 4 per cent eosinophils and 4 per cent monocytes Marked anisocytosis was noted with macro-ovalocytes and a slight increase in the number of neutrophilic polymorphonuclear leukocytes with hypersegmented nuclei An occasional Howell Jolly body was seen

Because of these findings a sternal bone marrow aspirate was obtained This showed an occasional overly large metamyelocyte and some lagging of maturation of the nucleus in some of the red cell precursors also a moderate eosinophilia

Unfortunately vitamin B administration was begun immediately on notification of the suspected diagnosis and serum was not obtained for vitamin B assay However urinary excretion testing revealed that only 1.13 per cent of an oral labeled dose of vitamin B was excreted this was raised to 11.0[illegible] per cent when the oral vitamin B_{12} dose was supplemented with hog intrinsic factor concentrate

The diagnosis of early addisonian pernicious anemia was made and the patient was discharged on maintenance injections of vitamin B_{12}.

It is of interest that the diagnosis could have been made two years earlier. In 1956 the patient had a single episode of Henoch Schonlein purpura with melena and microscopic hematuria. She was hospitalized throughout the course of that episode. Seven hematocrits ranged from 32 to 36 per cent and reticulocyte counts varied from 0.7 to 2.2 per cent. No red counts or hemoglobin determinations were made. A bone marrow aspirate was obtained and reported as showing "myeloid hyperplasia with eosinophilia" and nothing else of interest. However review of that marrow specimen at Montefiore Hospital in 1958 revealed the presence of large metamyelocytes and red cell precursors with more finely divided chromatin than normal (i.e. intermediate megaloblasts) such as were seen in the bone marrow aspirated in 1958.

Of further interest is the fact that when an episode of syncope occurred during the 1956 hospitalization, a thorough neurologic examination was performed. The findings were: "Vibration slightly depressed at ankles. Ankle jerks absent. Hypoaesthesia distal phalanx left middle finger. May have mild peripheral neuropathy, evidence equivocal."

Had the hematologic and neurologic findings been given the attention they deserved, the diagnosis of addisonian pernicious anemia would have been made two years earlier. Luckily, in this particular instance there was no marked progression of the neurologic damage during the period of delay, and no permanent irreversible damage was done. Incidentally, hypersegmented polymorphonuclear leukocytes and macrocytes were noted in the 1956 bone marrow specimen when it was reviewed two years later, and they were undoubtedly also present in the peripheral blood. In only one of the six peripheral blood smear reports obtained in 1956 was the presence of macrocytes noted (and ignored); the presence of hypersegmented polymorphonuclear leukocytes was not even mentioned.

The most prominent symptoms and signs of vitamin B_{12} deficiency disease are recorded in TABLES 3 AND 4, adapted from

TABLE 3—*Symptoms of Vitamin B*[illegible] *Deficiency Disease*

	Pre Liver Series		Edinburgh Series 1944–56 Davidson
	1908 Cabot Boston	*1923* Panton London	
No. of cases	1,200	11	250
Weakness and tiredness	90%	91%	90%
Dyspnoea	83%	—	[illegible]0%
Sore tongue	43% (of 3[illegible] cases)	[illegible]4% ([illegible]134 cases)	25%
Diarrhoea	51%	26%	9%
Constipation	35%	—	16%
Paraesthesiae	100%	—	38%

TABLE 4—*Signs of Vitamin B*[illegible] *Deficiency Disease*

	Pre Liver Series		Edinburgh Series 1944–56 Davidson
	1908 Cabot Boston	1923 Panton London	
No. of cases	1,200	11	250
Fever	9%	1	22%
Icterus	Most cases	—	Uncommon
Acute glossitis (red and ulcerated)	4[illegible]% (of 5[illegible] cases)	—	5%
Chronic atrophic glossitis (pale and smooth)	—	—	[illegible]
Splenomegaly	[illegible]	18	8%
Spinal cord damage	11	—	[illegible]%
Ataxic type		—	6%
Spastic type	4	—	1
Peripheral blood			
< [illegible] million R.B.C./c.mm.	[illegible]%	Average	58%
< 1 million R.B.C./c.mm.	4	1·39 million	1%

From DAVIDSON, S. Clinical picture of pernicious anaemia prior to introduction of liver therapy in 1926 and in Edinburgh subsequent to 1944. Brit. M. J. 1:241, 1957.

Sir Stanley Davidson's recent article on the differences in the clinical picture of pernicious anemia before and since the introduction of liver therapy[439] These differences are due to generally earlier diagnosis in those geographic areas where adequate

facilities for such early diagnosis exist. In those areas where such facilities either do not exist or are not used to advantage, the frequency of the various signs and symptoms of vitamin B_{12} deficiency approximates that observed in the preliver era.

The most frequent initial symptoms are weakness, dyspnea, paresthesia, and sore tongue, any or all of which may precede the onset of overt anemia by years.

An 82 year old woman had wandered from clinic to clinic for 12 years complaining of constant tingling in her hands, weakness, and occasionally a sore tongue and dyspnea. Infrequent hemoglobin determinations were always above 10 Gm./100 ml. of blood. The usual diagnosis made was "neurosis," and multivitamins (sometimes containing folic acid) were the usual therapy. When finally seen at the Bronx Municipal Hospital Center, she had gross generalized neurologic damage and marked megaloblastic anemia, with a hemoglobin of 4.6 Gm. She stated her skin color had been lemon yellow for at least two years. The diagnosis of vitamin B_{12} deficiency disease was made and confirmed by a serum vitamin B_{12} level of 70 μμg./ml. (L. Leishmannii assay).[453] Failure to absorb vitamin B_{12} was demonstrated by a modified Schilling test,[4] but it was not possible subsequently to determine whether the absorptive defect was correctible by exogenous intrinsic factor, because the patient developed vomiting and diarrhea and died in uremia associated with a chronic pyelonephritis after a period of rising BUN and impaired consciousness.

When this case was being prepared for presentation at a clinical pathologic conference, the records of the various clinics in which she had been seen during the preceding 12 years were examined. Each of the many times she was seen by a new doctor, comment was recorded of her complaints of paresthesia, weakness, and dyspnea. Not once was vitamin B_{12} deficiency considered. Yet persistent symmetrical paresthesias of the fingers (or toes) are a major clue to the possible existence of vitamin B_{12} deficiency, and thorough evaluation of this possibility is always indicated.[443]

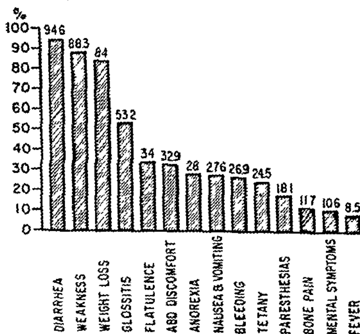
The folic acid deficiency states present a similar clinical picture to that of vitamin B_{12} deficiency, with the exceptions noted at the beginning of this chapter. Pure folic acid deficiency is rarely seen, and the actual clinical picture usually observed is that of folic acid deficiency combined with other deficiencies

and often associated with gross disorder of gastrointestinal function

In the malabsorption syndrome the most frequent symptoms are diarrhea, weakness and weight loss, all of which occur in more than 4 of every 5 patients.[4] Glossitis occurs in approximately half the cases, paresthesia in almost 20 per cent, and mental symptoms in about 10 per cent. The neurologic symptoms are possibly due in large part if not completely to associated vitamin B_1 deficiency.

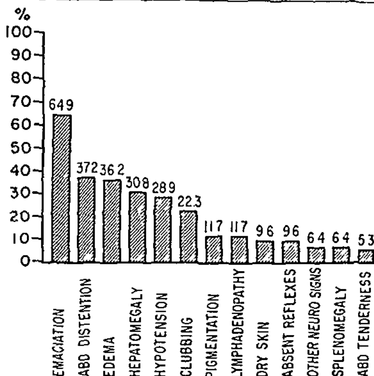
The major symptoms and signs of the malabsorption syndrome are noted in TABLE 5 AND 6, adapted from a presenta

TABLE 5 — Major Symptoms of the Malabsorption Syndrome



From Bossak, E. T., Wang, C. I., and Adlersberg, D.: Clinical aspects of the malabsorption syndrome (idiopathic sprue). J. Mount Sinai Hosp. (New York) [illegible] 86 [illegible]. Simultaneously published in Adlersberg, D., Ed.: The Malabsorption Syndrome. New York, Grune and Stratton, 19[illegible].

TABLE 6—*Major Signs of the Malabsorption Syndrome*



From Bo sak F T Wang C I and Adlersberg D Clinical aspects of the malabsorption syndrome (idiopathic sprue) J Mount Sinai Hosp (New York) 24 286 1957 Simultaneously published in Adlersberg D Ed The Malabsorption Syndrome New York Grune and Stratton 1957

tion [4 4] at a recent symposium on the malabsorption syndrome held at the Mount Sinai Hospital New York

The clinical picture in those conditions other than the vitamin B_1 or folic acid deficiency states which are sometimes associated with a megaloblastic marrow is that of the underlying condition (hemolytic anemia etc)

Pernicious anemia is associated with an increased incidence of carcinoma of the stomach as noted in the section Lesions which *Destroy the Gastric Mucosa* and this entity must be

looked for in every patient with addisonian pernicious anemia It is also of importance that every patient with cancer of the stomach be evaluated for possible vitamin B_1 deficiency caused by inability to produce intrinsic factor

The incidence of gall bladder disease in patients with pernicious anemia is considerably greater than chance [4, 5-457] This may be due in large part to the fact that pernicious anemia is a hemolytic anemia in addition to being an anemia with inadequate production of circulating erythrocytes [4, 4, 8] In pernicious anemia there is an increased random destruction of not only the patient s own erythrocytes but also of transfused normal red cells [4, 9] The resulting hyperbilirubinemia leads to an increased concentration of bilirubin in the bile with a large amount of bile pigment from sources other than the mature circulating erythrocytes [460] perhaps arising mainly from rapidly destroyed young erythrocytes [46] This in turn may lead to the precipitation of pigment in the gall bladder cholelithiasis and cholecystitis [461, 461]

The serum bilirubin of untreated patients with addisonian pernicious anemia is generally in the range of 1 mg 100 ml [46] and may or may not be of a degree sufficient to produce the light lemon yellow skin coloration classically associated with untreated pernicious anemia

Clear-cut relationships between pernicious anemia per se and diseases other than those already mentioned have not yet been established but may exist One such disease is gouty arthritis [46] It is obvious however that the fortuitous presence of another disease can alter the usual clinical picture of any of the megaloblastic anemias

Acute liver destruction such as occurs with acute hepatitis may completely mask vitamin B_1 deficiency disease [463, 464] The liver damage releases residual vitamin B_1 stores into the blood stream [465] and apparently thereby makes them available for hematopoiesis

7 *Differential Diagnosis*

I CLINICAL

THE SINGLE MOST USEFUL TOOL in separating the megaloblastic anemias is an adequate history This should include detailed inquiry into the quantity of animal protein and fresh green vegetables consumed in an average week The author has seen the comment diet adequate on the charts of 3 patients with megaloblastic anemia of obscure etiology Conversation with the patients revealed that 2 of them had substituted alcohol for almost all solid food for periods in excess of a year and the third was a strict vegetarian! The first 2 had dietary folic acid deficiency associated with chronic alcoholism and cirrhosis the third had dietary vitamin B_{12} deficiency The proper diagnosis was confirmed by appropriate tests and response to therapy in all 3 patients

Familiarity with the most frequent causes of megaloblastic anemia in various geographic areas helps in differential diagnosis Every physician should be familiar with the most common causes of megaloblastic anemia in his own area For example in the New York metropolitan area the two most common causes of megaloblastic anemia are pernicious anemia and the malabsorption syndrome in their various subdivisions (see CHAPTER 4) The malabsorption syndrome is the most common cause of megaloblastic anemia in that large segment of the New York City population composed of recent migrants from Puerto Rico pernicious anemia is more common in the rest of the New York population generally

Age is important pernicious anemia is very uncommon under the age of 40 (see CHAPTER 1) [14] Origin of the patient should be ascertained persons from the Scandinavian countries are more likely to have pernicious anemia or fish tapeworm anemia those from England Ireland and Canada have a greater chance of having pernicious anemia patients from India may have nutritional vitamin B_{12} deficiency because of religious dietary restric

tions (those with nutritional folic acid deficiency due to dietary inadequacy because of poverty are less likely to travel)

The presence of nervous system involvement indicates vitamin B_{12} deficiency Nervous system damage due solely to folic acid deficiency with concomitant vitamin B_{12} deficiency adequately excluded has never been described in the literature to the author s knowledge and the author has never seen such a case In the presence of a megaloblastic anemia barring the fortuitous presence of unrelated neurologic disease nervous system involvement indicates vitamin B_{12} deficiency with or without concomitant folic acid deficiency It never indicates folic acid deficiency alone This delineation is so clear that it has been suggested that neurologic damage associated with megaloblastic anemia be referred to as vitamin B_{12} neuropathy [40]

Each of the symptoms and signs of nervous system involvement mentioned in CHAPTER 5 should be looked for carefully since only one or two may be present This is especially true in vitamin B_{12} deficiency without overt megaloblastic anemia when only careful examination of the peripheral blood smear suggests the diagnosis

If the patient has been taking multivitamins containing folic acid especially if the amount of folic acid in them is in excess of 0 50 mg per day the peripheral blood picture may be almost or even completely normal It is quite possible that the lack of parallelism between the degree of hematopoietic and nervous system damage in vitamin B_{12} deficiency states is primarily a function of the highly variable dietary folic acid ingestion (and possibly absorption as well) from patient to patient Regardless of diet however the author has never seen a patient with vitamin B_{12} neuropathy not taking multivitamins who did not have macroovalocytes and hypersegmented polymorphonuclear leukocytes in the peripheral blood

Diarrhea strongly suggests the malabsorption syndrome and folic acid deficiency often accompanied by vitamin B_{12} deficiency However it should be remembered that diarrhea also occurs not infrequently in pernicious anemia [443] Conversely the

absence of diarrhea rules against the malabsorption syndrome but does not exclude it[46] *

This is especially true of adult coeliac disease of which many cases have been described with bowel movements appearing normal in both quality and quantity[384 4 4 446 46] Some of these cases had macrocytosis in a few a megaloblastic marrow was described The author has seen 2 patients with megaloblastic anemia related to occult adult coeliac disease Both had single daily normal formed stools no history of diarrhea low serum vitamin B_1 and folic acid levels intrinsic factor in their gastric juice by in vitro assay and inability to absorb an oral test dose of radioactive vitamin B_1 either without or with added hog intrinsic factor concentrate In both balance studies revealed defective fat absorption and produced steatorrhea In both patients the megaloblastic anemia disappeared after intramuscular vitamin B_{12} administration and oral folic acid therapy Similar cases including one with only partial remission after combined vitamin B_1 and folic acid administration were reported prior to the wide availability of serum vitamin B_1 determinations and description of the Schilling test[384]

In pregnant women and infants folic acid deficiency is by far the most likely cause of megaloblastic anemia

When the usual signs and symptoms of vitamin B_1 or folic acid deficiency are lacking more obscure causes of megaloblastic like anemia must be sought These include hidden malignancies especially leukemia and hemolytic anemias of various types

Thorough knowledge of the information contained in CHAPTERS 4 and 5 will make differential diagnosis of the megaloblastic anemias a relatively simple matter for the careful clinician In areas where laboratory confirmation of the diagnosis is unavailable therapeutic *trial constitutes the time honored method of* differentiating the folic acid deficiency states from those due to vitamin B_{12} deficiency[444]

Therapeutic trial carries with it the danger that either vitamin B_1 or folic acid may produce a temporary remission in

patients whose primary deficiency is of the other agent thus leading the physician into improper therapy (see CHAPTER 8) For this reason only a single small dose or single small daily doses should be given to the patient in such a trial

The patient should be maintained on a diet low in folic acid and vitamin B_1 for at least two days prior to the test (preferably for a week) and throughout the test period A single 1 μg injection of vitamin B_1 is then given and the reticulocyte count observed daily or at least every other day for seven to 12 days[468] If there is no reticulocytosis of significance the patient is next given 0 5 mg of folic acid subcutaneously and reticulocytosis sought as previously

Daily doses of 0 4 mg of folic acid for 10 days have proved acceptable in therapeutic trials Such a course of therapy induces hematologic response only in patients with folic acid deficiency[468] More study of the relative value of a single small dose of vitamin B_{12} or folic acid vs daily small doses in therapeutic trials is needed Daily small doses may be more reliable

Variants of the procedure using similar oral rather than parenteral small doses of each vitamin may be used to test absorption provided the status of the patient is good enough to allow the time to carry out peroral studies of about one week each prior to parenteral vitamin administration (Peroral studies should be performed first because parenteral administration of the appropriate vitamin will always produce a greater reticulocyte response and the method of serial reticulocyte responses to test agents should be carried out with the weaker agent given first One μg of vitamin B_1 given by mouth is less potent than the same dose parenterally because even in a completely normal subject complete absorption from the gastrointestinal tract is rare the same is true of oral versus parenteral doses of folic acid)

II LABORATORY[468b]

A Gastric Acidity

The most widely used laboratory aid to the separation of the megaloblastic anemias is the determination of gastric acidity[469]

If the patient is over the age of 50 the presence of free acid *almost* rules out pernicious anemia completely if he is under 50 then the presence of free acid makes pernicious anemia less likely but does not rule it out entirely [131, 133]

Achlorhydria in a patient past the age of 40 is of only moderate significance in terms of the possibility of pernicious anemia Approximately 15 per cent of people between the ages of 40 and 60 have histamine refractory achlorhydria as do about 25 per cent of individuals between ages 60 and 70 and 30 per cent of persons over the age of 70 [470]

Since intubation of the patient is unpleasant tubeless gastric analysis has become popular [471, 472] While this method for determination of gastric acidity is simple it is unreliable in the presence of renal disease gastric obstruction diarrhea and malabsorption syndrome [473] as well as in gastrectomized patients and individuals with a rapid gastric emptying time

Tubeless gastric analysis involves the ingestion by the patient of a cation exchange resin to which is bound quininium azure A or another cation If there is free acid in the stomach the hydrogen ions will replace the cation on the resin which will then be absorbed into the blood stream and released in the urine where its quantity may be measured and thereby provide a measure of the amount of hydrogen ions (i e of free acid) in the stomach Unfortunately cations in the small intestine will also exchange with the cation on the resin making it important to examine only the urine voided within two hours of ingestion of the resin which will contain only cation which was released in the stomach However if the patient has a fast gastric emptying time even the urine voided within two hours of ingestion of the resin may contain cation released in the small intestine and an erroneous impression of free acid in the stomach may be given

Unexplained results positive for free acid have occurred in the presence of achlorhydria proven by intubation frequently enough to make the azure A resin test of dubious value in the detection of diseases associated with achlorhydria [474] The author

has seen 2 patients with pernicious anemia in whom the correct diagnosis was "ruled out" by an intelligent house staff because the presence of free acid was reported on tubeless gastric analysis with azure A resin. The diagnosis was "ruled back in" by insisting on intubation and demonstrating the absence of free acid in the gastric aspirate. It is unknown whether the erroneous results were due to an error in technic, such as chewing of the resin granules by the patients or collection of the urine specimen more than two hours after ingestion of the resin.

Although tubeless gastric analysis using azure A resin cannot at present be recommended, the older tubeless method, using a quininium resin, appears to be more reliable and may be a useful screening procedure.[473, 474]

B. Assay of Intrinsic Factor in Gastric Juice

In vivo assay of intrinsic factor content of gastric juice is accomplished by taking the gastric aspirate from a donor (the patient) and giving it to a recipient who is known to have no intrinsic factor, along with a small dose of radioactive vitamin B_{12}, in a Schilling type test (see C below). Alternatively, the donor's gastric juice may be given to an untreated patient with known pernicious anemia along with 1 μg of vitamin B_{12}. A hematologic response of the recipient, unless due to a fortuitous spontaneous remission, would rule out lack of intrinsic factor in the donor.

In vitro "assay" of intrinsic factor in human gastric juice has been reported recently.[475, 476] This procedure obviates the need for more than a few ml. of gastric juice from the patient and also eliminates the need for a recipient. In the author's hands, when the gastric juice has been frozen immediately after collection, this "assay" has proved generally accurate as an indication of the presence or absence of intrinsic factor in the gastric juice of achlorhydric patients. Great care in interpretation of results is required, especially in the presence of mucus (including salivary mucus), which binds vitamin B_{12},[477, 478] or of bile, which may contain nonradioactive vitamin B_{12}.[479, 480] Free acid and

If the patient is over the age of 50 the presence of free acid almost rules out pernicious anemia completely if he is under 50 then the presence of free acid makes pernicious anemia less likely but does not rule it out entirely[131 133]

Achlorhydria in a patient past the age of 10 is of only moderate significance in terms of the possibility of pernicious anemia Approximately 15 per cent of people between the ages of 40 and 60 have histamine refractory achlorhydria as do about 25 per cent of individuals between ages 60 and 70 and 30 per cent of persons over the age of 70[470]

Since intubation of the patient is unpleasant tubeless gastric analysis has become popular[471 47] While this method for determination of gastric acidity is simple it is unreliable in the presence of renal disease gastric obstruction diarrhea and malabsorption syndrome[473] as well as in gastrectomized patients and individuals with a rapid gastric emptying time

Tubeless gastric analysis involves the ingestion by the patient of a cation exchange resin to which is bound quininium azure A or another cation If there is free acid in the stomach the hydrogen ions will replace the cation on the resin which will then be absorbed into the blood stream and released in the urine where its quantity may be measured and thereby provide a measure of the amount of hydrogen ions (i e of free acid) in the stomach Unfortunately cations in the small intestine will also exchange with the cation on the resin making it important to examine only the urine voided within two hours of ingestion of the resin which will contain only cation which was released in the stomach However if the patient has a fast gastric emptying time even the urine voided within two hours of ingestion of the resin may contain cation released in the small intestine and an erroneous impression of free acid in the stomach may be given

Unexplained results positive for free acid have occurred in the presence of achlorhydria proven by intubation frequently enough to make the azure A resin test of dubious value in the detection of diseases associated with achlorhydria[474] The author

ducible in any one laboratory (see TABLES 7 AND 8) Determination of whether a given result represents a low serum vitamin B_{12} level should be based on normal controls in the same laboratory not on results in other laboratories

Ochromonas malhamensis is the most specific assay organism Euglena gracilis is next in specificity first in sensitivity Lactobacillus leishmanii and Escherichia coli are less specific and sen

TABLE 7—*Serum Vitamin B_{12} Concentrations in Normal Subjects*

Author	Date	Number of subjects	Serum B_{12} ($\mu\mu g$/ml)		Test organism
			Range	Mean	
Ross	1950	17	300 to [illegible]0	490	Euglena
Mollin and Ross	1952 4	1[illegible]6	100 to 900	300	Euglena
Heinrich and Lahann	1953 4	—	6 to 460	238	Euglena
Killander	1953	56	100 to [illegible]0	360	Euglena
Lear et al	1954	[illegible]0	299 to 856	[illegible]	Euglena
[illegible] and Beard	1954	57	86 to 460	21[illegible]	Euglena
Rosenthal and Sarett	19[illegible]	24	80 to 4[illegible]0	[illegible]00	L leichmannii
Girdwood	1954	[illegible]0	0 to 8[illegible]0	3[illegible]0	L leichmannii
Nieweg et al	19[illegible]	[illegible]	310 to 10[illegible]0	—	L leichmannii
Okuda et al	1954	33	—	1[illegible]9	L leichmannii
Unglaub et al	1954	—	0 to 4[illegible]0	10	L leichmannii
[illegible]ger et al	1955	5[illegible]	0 to 1060	60	L leichmannii
Spray	19[illegible]	111	1[illegible]0 to 860	43[illegible]	L leichmannii
Grossowicz et al	19[illegible]	90	[illegible]00 to 1000	—	E coli

Taken from MOLLIN D L AND ROSS G I M (see TABLE 8 page 8[illegible])

sitive but have the advantage of rapid growth They provide assay results in a day as compared to a week for Ochromonas and Euglena

The more specific organisms are generally preferable Occasionally a less specific organism will grow on factors other than vitamin B_{12} contained in the serum of a vitamin B_{12} deficient patient thereby conveying the erroneous impression that the patient is not deficient in the vitamin We[51] have seen a patient with proved vitamin B_{12} deficiency (cerebral and hematologic involvement classic Schilling test result) whose serum

pepsin release nonradioactive vitamin B_1 from the liver thereby producing false low results The assay using liver homo genate [4 0] rather than liver slices [47] has proven especially suscep tible to errors introduced by these various factors and cannot at present be recommended Other sources of error in both assays include the variable amount of vitamin B_1 in gastric juice (from less than 40 μμg /ml to more than 1500 μμg /ml in our laboratory) and the variable amount of what may be de graded intrinsic factor in the gastric juice This latter material appears able to block the receptors for intrinsic factor on liver but it appears unable to then bind vitamin B_1 [1 3]

Evaluation in other laboratories will indicate any general usefulness of the in vitro procedure So far it seems to be specific for intrinsic factor In vitro assay procedures for intrinsic factor must be evaluated very cautiously The first such proce dure [481] proved to be nonspecific [564] and to measure only vitamin B_1 binding [06] a property common to many substances That procedure conveyed the erroneous impression that intrinsic factor is lacking in patients with sprue to an even greater extent than in patients with pernicious anemia [482] In point of fact only those patients with sprue who also have gastric atrophy have a lack *of intrinsic factor as a significant etiologic factor in their* megaloblastic anemia [3] Numerous observers have demonstrated intrinsic factor to be present usually in the gastric juice of pa tients with malabsorption syndrome by in vivo assay [115 1 8 173 245]

C Vitamin B_1 Serum Levels

Microbiologic assay of serum vitamin B_1 concentration is rather widely available in large medical centers but not yet elsewhere The procedure is extremely useful as an objective estimate *of the degree of vitamin B_1 deficiency* [3 4 3]

A number of different assay microorganisms with varying degrees of specificity for vitamin B_1 have been used to deter mine serum vitamin B_1 concentrations [0 64 4 4 3 483 48] Re sults vary in different laboratories but are generally repro

level falls first and is slowly followed by the red cell vitamin B_{12} level. Thus in 5 patients whose serum vitamin B_{12} levels had fallen to an average of 35 $\mu\mu g$/ml the vitamin B_{12} content of the unwashed red cells still averaged 110 $\mu\mu g$/ml.[487] Further studies are needed to define the possible usefulness of determining red cell vitamin B_{12} levels as a measure of general tissue depletion of the vitamin. In such studies it must be kept in mind that red cell vitamin B_{12} levels may include a large fraction of vitamin B_{12} contained in leukocytes.

In early vitamin B_{12} deficiency prior to the development of anemia and a clearly megaloblastic bone marrow, and after the appearance of macroovalocytes and an increase in number of hypersegmented neutrophilic polymorphonuclear leukocytes in the peripheral blood, serum vitamin B_{12} levels frequently have not fallen below the range of normal, although they are always well below the mean normal value.[488]

D. Folic Acid Serum Levels

Microbiologic assay of blood folic acid has fallen into disrepute since the demonstration that such assay does not measure folic acid but rather measures a multiplicity of factors, all of which have folic acid like activity for microorganisms but none of which is folic acid.[88, 89] Although folic acid per se is not normally present in serum, it is accepted that what is measured in serum immediately after administration of folic acid orally or parenterally is folic acid itself. Theoretically, however, the only valid measure of folic acid depletion would be direct assay of tissue stores of this vitamin as both folic and folinic acids.[31, 39] The next best measure would be assay of serum folic acid levels if such could be determined. Other laboratory procedures, such as the indirect methods discussed in the section "Tests of Folic Acid Absorption, Stores and Utilization" and the section "Assay of Metabolic Products Found in the Urine of Folic Acid Deficient Patients," must be interpreted with extreme caution. It must especially be kept in mind that since vitamin B_{12} deficiency results in deranged folic acid metabolism, the metabolic prod

vitamin B_{12} level was only 20 $\mu\mu g$/ml by E. gracilis assay yet with a serum vitamin B_{12} level of 1,[illegible]00 $\mu\mu g$/ml by L. leishmanii assay. The result was thrice repeated prior to treatment of the patient. A similar case has been observed by Lowenstein.[48]

The value of determining "free" B_{12} as well as "total" B_{12} has not been established. "Free" B_{12} is that quantity of the vitamin in human serum which is available to the assay microorganism

TABLE 8—*Serum Vitamin B_{12} Concentrations in Pernicious Anemia in Relapse*

Author	Date	Number of patients	Serum B_{12} ($\mu\mu g$/ml)		Test organism
			Range	Mean	
Mollin and Ross	19[illegible]2–4	100	<16 to 105	40	*Euglena*
Heinrich and Lahann	19[illegible]3–4	—	0.01 to 30	[illegible]	Euglena
Killander	19[illegible]3	12	<25 to 105	38	Euglena
Lear et al	19[illegible]4	3[illegible]	0 to 8[illegible]	39	Euglena
[illegible]itney and Beard	19[illegible]	1[illegible]	0 to [illegible]	13	Euglena
Girdwood	1954	36	<50 to 200	95 (25) <50 (11)	L. leichmannii
Nieweg et al	1954	1[illegible]	<[illegible] to 1[illegible]	—	L. leichmannii
Unglaub et al	1954	10	10 to [illegible]0	40	L. leichmannii
Spray	1955	17	[illegible] to 110	—	L. leichmannii
Grossowicz et al	19[illegible]	8	50—130	[illegible]5	*E. coli*

From MOLLIN, D. L., AND ROSS, G. I. M.: The pathophysiology of vitamin B_{12} deficiency in the megaloblastic anaemias. *In* Heinrich, H. C.: Vitamin B_{12} und Intrinsic Factor. Stuttgart, Ferdinand Enke, 1957.

prior to the routine heating of the serum to release B_{12} from serum protein. The amount of "free" B_{12} in a given sample of serum is different for different microorganisms and different durations of incubation. The physiologic significance in man of "free" B_{12} is unknown although it is generally believed that it represents vitamin B_{12} not bound to protein or to any other substance.

Recent evidence[486, 487] indicates that in the normal individual the serum and red blood cell vitamin B_{12} levels are roughly equal. As vitamin B_{12} deficiency develops the serum vitamin B_{12}

E Tests of Folic Acid Absorption Stores and Utilization

Measurable amounts of folic acid appear in the serum of individuals given oral doses of 1 mg or more of the vitamin [4, 31, 490–49]

Amounts of folic acid averaging less than 10 μg daily are normally excreted in the urine [493–49] When folic acid is administered orally or parenterally urinary excretion is markedly increased [336, 493, 495, 496]

These observations have been used to advantage in studying folic acid absorption stores and utilization [4] Following the subcutaneous administration of 5 mg of folic acid the urinary excretion of less than 1 5 mg in 24 hours is an indication of gross tissue depletion [336] The converse is not necessarily true (i e urinary excretion of more than 1 5 mg does not mean the patient is not desaturated) perhaps because of an obligatory excretion by the kidney [9]

A similar indirect measure of the tissue stores of folic acid is determination of the rate of clearance of an intravenous dose of 15 μg of folic acid per kg of the patient s body weight [497] This dose appears to be completely cleared after 15 minutes in nearly all patients who are clinically deficient in folic acid but not in normal control subjects Of course since this test measures the end result of a number of phenomena rapid clearance of a test dose of folic acid can only be considered suggestive of folic acid deficiency and not conclusive for it This is equally true for any indirect test of folic acid stores

After saturation of the tissues by administration of 15 mg of folic acid subcutaneously for a week Girdwood [336] has determined the urinary excretion of a 5 mg oral dose He found that the excretion of less than 1 5 mg of folic acid in the subsequent *24 hour period was an indication of severe malabsorption*

While these results have been confirmed by Cox et al [4, 8] other workers found sufficient overlap in urinary excretion after the test dose in saturated normal subjects and patients with malabsorption syndrome to make interpretation of the results difficult [31] They proposed saturation of the folic acid-deficient

ucts of folic acid deficiency may be found in the urine of patients who have adequate ingestion and absorption of folic acid but utilize it inadequately due to vitamin B_{12} deficiency. Failure to recognize this fact may lead to an erroneous diagnosis of primary folic acid deficiency when in fact the patient lacks only vitamin B_{12}. Similarly, injected or orally administered folic acid may rapidly disappear into the tissues of patients with vitamin B_{12} deficiency, and such rapid disappearance in these cases does not indicate a primary folic acid deficiency.[4]

Because of these problems of interpretation of indirect methods of assaying folic acid deficiency states, we[489, 489a, 489b] decided to investigate the possibility that the folic acid-like activity in human serum may in fact reflect the presence or absence of primary folic acid deficiency.

We have so far determined the serum "folic acid" levels of more than 100 patients with megaloblastic anemia using a modified L. casei assay technic which is simple and rapid (the organism grows well within 24 hours)' Low levels (below 5 mµg of folic acid activity per ml. of serum) were found in those sera from patients proved to have primary folic acid deficiency by history, inadequate response to vitamin B_{12} therapy and/or normal serum vitamin B_{12} levels, and excellent response to folic acid administration. Those sera from patients with primary vitamin B_{12} deficiency proved by low serum vitamin B_{12} levels and excellent hematologic response to vitamin B_{12} therapy had "folic acid" levels above 7 mµg/ml.[489] Some patients with primary deficiencies of both vitamins were uncovered; these patients proved to be ingesting grossly inadequate diets (often while pregnant) and/or to be suffering from malabsorption syndrome. All sera were obtained while the patients were fasting.

It is the author's belief that determination of the serum "folic acid" level may prove to rank with assay of the serum vitamin B_{12} level as a prime laboratory tool in evaluation of all patients with megaloblastic anemia.

after a loading dose of 5 Gm of l histidine These patients were proved to be folic acid deficient by presence of low serum folic acid levels normal serum vitamin B_1 levels and excellent hematologic response to folic acid therapy after inadequate responses to vitamin B_1 injection Because of this and because of the necessity for at least one and frequently more than one 24 hour urine collection (difficult to obtain from infants and incontinent adults) to study for FIGlu we do not use FIGlu assay routinely in evaluation of patients with megaloblastic anemia at present

Using the more precise enzymatic method Hiatt did not observe FIGlu in the urine of a patient proved by therapeutic trial to have folic acid deficiency by Marshall Jandl and Castle FIGlu did not appear after a 2 Gm loading dose of l histidine (personal communication) This result adds another case to the accumulating series of patients with proved folic acid deficiency who nonetheless do not excrete FIGlu in their urine [09 1]

It must be remembered that severe vitamin B_1 deficiency by interfering with folic acid metabolism may induce FIGlu excretion [513] * *This may lead to a false diagnosis of primary folic* acid deficiency (i e inadequate ingestion or absorption of folic acid) when in fact the only primary deficiency may be in the amount of vitamin B_1 in the patient s body and the patient may have only *a secondary deficiency of folic acid (i e inade*quate utilization of an adequate supply of folic acid due to insufficient vitamin B_{12} for normal folic acid metabolism)

Recent studies [14 514] indicate that in folic acid deficient animals formic acid possibly derived from tryptophane metabolism also appears in the urine in high concentration Assay of the concentration of this agent in urine may also prove of some use as an index of folic acid deficiency again keeping in

* Patients with vitamin B deficiency *and no determinable folic acid deficienc* who nevertheless excreted FIGlu in their urine have been observed by Rucknagel LaDu Laster Seegmiller Daft and Silverman Marshall Jandl Castl and Hiatt and Herbert Baker Frank Pasher Wasserman and Sobotka

patient with 20 to 10 mg of folic acid for at least four weeks followed by oral administration of a 3 mg test dose and assay of the serum folic acid concentration one two and four hours later In their studies peak serum concentrations in normal subjects ranged from 42 to 170 μmg of folic acid per ml of serum Peak values below 40 μmg were found almost uniformly in patients with malabsorption syndrome

Caution in interpreting any of these indirect tests of folic acid deficiency is warranted to avoid making an erroneous diagnosis of primary folic acid deficiency when the patient actually is suffering from a primary vitamin B_1 deficiency[4] In severe vitamin B_1 deficiency the disappearance of ingested or injected folic acid appears to be as rapid as it is in folic acid deficiency[4, 497]

F Assay of Metabolic Products Found in the Urine of Folic Acid Deficient Patients

Normally histidine is metabolized to N formimino L glutamic acid (FIGlu) which is degraded to glutamic acid in the presence of folic acid[499, 00] Folic acid deficiency either primary or secondary results in excretion of undegraded FIGlu in the urine[01] this metabolic defect may be made more striking by feeding l histidine[0]

When folic acid deficiency is very severe high concentrations of unmetabolized FIGlu may be found in the urine[03, 514] In milder folic acid deficiency states it may be necessary to give the patient l histidine orally to induce FIGlu excretion in the urine[09, 11] Thus assay of the amount of FIGlu in the urine by microbiologic[04, 09] or enzymatic and spectrophotometric methods[06, 07] may prove to be a useful measure of folic acid deficiency

Using microbiologic methods[04, 09] we[1] have not been able without giving the patients oral load doses of l histidine to find FIGlu in the urine of some patients with megaloblastic anemias of infancy pregnancy poor diet and malabsorption syndrome In some of these cases we did not find FIGlu in the urine even

after a "loading" dose of 5 Gm of l-histidine. These patients were proved to be folic acid deficient by presence of low serum folic acid levels, normal serum vitamin B_{12} levels, and excellent hematologic response to folic acid therapy after inadequate responses to vitamin B_{12} injection. Because of this and because of the necessity for at least one and frequently more than one 24-hour urine collection (difficult to obtain from infants and incontinent adults) to study for FIGlu, we do not use FIGlu assay routinely in evaluation of patients with megaloblastic anemia at present.

Using the more precise enzymatic method, Hiatt did not observe FIGlu in the urine of a patient proved by therapeutic trial to have folic acid deficiency by Marshall, Jandl, and Castle. FIGlu did not appear after a 2 Gm "loading" dose of l-histidine (personal communication). This result adds another case to the accumulating series of patients with proved folic acid deficiency who nonetheless do not excrete FIGlu in their urine.[509-1]

It must be remembered that severe vitamin B_{12} deficiency, by interfering with folic acid metabolism, may induce FIGlu excretion.[513]* This may lead to a false diagnosis of primary folic acid deficiency (i.e., inadequate ingestion or absorption of folic acid) when in fact the only primary deficiency may be in the amount of vitamin B_{12} in the patient's body, and the patient may have only a secondary deficiency of folic acid (i.e., inadequate utilization of an adequate supply of folic acid due to insufficient vitamin B_{12} for normal folic acid metabolism).

Recent studies[514, 515] indicate that in folic acid-deficient animals formic acid, possibly derived from tryptophane metabolism, also appears in the urine in high concentration. Assay of the concentration of this agent in urine may also prove of some use as an index of folic acid deficiency, again keeping in

* Patients with vitamin B_{12} deficiency and no determinable folic acid deficiency who nevertheless excreted FIGlu in their urine have been observed by Rucknagel, LaDu, Laster, Seegmiller, Daft and Silverman, Marshall, Jandl, Castle and Hiatt, and Herbert, Baker, Frank, Pasher, Wasserman, and Sobotka.

mind the caution that in severe vitamin B_{12} deficiency folic acid metabolism may be disturbed

G Tests of Vitamin B_1 Absorption

Absorption of vitamin B_1 may be measured following the oral administration of physiologic (two μ_g or less) doses of radioactive cobalt labeled vitamin [3] This may be done by determining the amount of radioactivity in the feces (Heinle Welch test) [515] the plasma [1] the liver (Glass test) [17] or the urine (Schilling test) [4 1] The relative merits of these four methods have recently been carefully appraised [3]

In terms of ease speed of execution and minimal retention of radioactivity by the patient the clinically most useful test at the present time is measurement of the percentage of the oral dose subsequently found in the urine this is generally known as the Schilling test after its originator [4 1] Plasma radioactivity determination is simpler but radioactive cobalt labeled vitamin B_1 of high enough specific activity for accurate measurement in the conventional well type counter of a plasma sample is not widely available

The author prefers a modified form [4 0 18] of the Schilling test because the original test requires a longer waiting period between test doses and a two hour delay between oral and parenteral doses of vitamin B_1 which reduces the adaptability of the test for outpatients [4 19] Additionally a single 24 hour urine collection following a single intramuscular injection of vitamin B_1 as a flushing dose may occasionally lead to mis diagnosis Patients with renal disease have delayed vitamin B_1 excretion and the first 24 hour urine collection may be mis leadingly low [0 1] The author has observed delayed excretion of radioactive vitamin B_1 occasionally in the absence of any determinable renal disease The most striking case in this regard was a 37 year old woman with transverse myelitis in whom neu ropathy due to vitamin B_1 deficiency was suspected and in whom determination of the serum B_1 level was valueless due to recent B_1 therapy She excreted 1 2 per cent of the oral dose

in the first 24 hours and 12 per cent in the second 24 hour period Had a single 24 hour collection been made the erroneous conclusion of *inability to absorb vitamin B_1 would have* been made (In a repeat test this patient displayed a normal excretion pattern The cause of the delayed excretion in the first test is unknown)

The 2 µg oral dose of radio B_1 as originally proposed by Schilling[4 1] is probably preferable to smaller doses It appears to just saturate the physiologic intrinsic factor dependent system for B_1 absorption which lesser doses do not[518] This saturation results in more highly reproducible tests than those with smaller doses which are affected more by small changes (such as mixture with small amounts of nonradioactive vitamin B_1 in the stomach and small intestine)

Oral radio B_1 doses in excess of 2 µg invalidate the Schilling test as a measure of the physiologic intrinsic factor dependent mechanism of vitamin B_1 absorption A significant aliquot of such supraphysiologic doses (i e doses much greater than the amount of vitamin B_1 in a well balanced meal) may be absorbed *independently of the intrinsic factor dependent system* (see page 20)

The modified Schilling test is performed as follows[4 0 518] 2 µg of radioactive *cobalt labeled* vitamin B_1 is given to the patient by mouth separated from food by at least two hours One mg of nonradioactive vitamin B_1 is injected intramuscularly immediately after the oral dose of labeled vitamin All urine is collected in the ensuing 24 hour period A second flushing injection of 1 mg nonradioactive vitamin B_1 is given to wash much of the remaining labeled vitamin out of the body and again the urine is collected for 24 hours The radioactivity in the two urine collections is determined in an appropriate radioactivity counting apparatus and recorded as per cent of the oral dose

If desired the radioactivity may be concentrated by evaporation or more difficult methods[3] prior to counting The evaporation procedure[5] consists in thorough mixing of the en

mind the caution that in severe vitamin B_1 deficiency folic acid metabolism may be disturbed

G Tests of Vitamin B_1 Absorption

Absorption of vitamin B_1 may be measured following the oral administration of physiologic (two μg or less) doses of radio active cobalt labeled vitamin[3] This may be done by determining the amount of radioactivity in the feces (Heinle Welch test)[15] the plasma[16] the liver (Glass test)[1] or the urine (Schilling test)[4,3] The relative merits of these four methods have recently been carefully appraised[3]

In terms of ease speed of execution and minimal retention of radioactivity by the patient the clinically most useful test at the present time is measurement of the percentage of the oral dose subsequently found in the urine this is generally known as the Schilling test after its originator[451] Plasma radioactivity determination is simpler but radioactive cobalt labeled vitamin B_1 of high enough specific activity for accurate measurement in the conventional well type counter of a plasma sample is not widely available

The author prefers a modified form[4,0, ,18] of the Schilling test because the original test requires a longer waiting period between test doses and a two hour delay between oral and parenteral doses of vitamin B_1 which reduces the adaptability of the test for outpatients[4, ,19] Additionally a single 24 hour urine collection following a single intramuscular injection of vitamin B_1 as a flushing dose may occasionally lead to mis diagnosis Patients with renal disease have delayed vitamin B_1 excretion and the first 24 hour urine collection may be mis leadingly low[,0, ,1] The author has observed delayed excretion of radioactive vitamin B_1 occasionally in the absence of any determinable renal disease The most striking case in this regard was a 37 year old woman with transverse myelitis in whom neu ropathy due to vitamin B_1 deficiency was suspected and in whom determination of the serum B_1 level was valueless due to recent B_1 therapy She excreted 1 2 per cent of the oral dose

TABLE 9 —*Range of Urinary Excretion of an Oral Dose of μg of Radioactive Vitamin B Measured as Per Cent of the Oral Dose*

Day	Oral Treatment	Normals	Pernicious Anemia	Sprue
1	Vitamin B alone	4 1–29 2	0 0– 2 7	0 1–2 2
		0 ,– 0 0	0 0– 1 5	0 1–0 6
3	B +Intrinsic Factor	6–29 4	2 0–16 4	0 –1 5
4		1 4–12 8	0 1–10 3	0 1–1 1

TABLES 9 AND 10 delineate the range and average of the urinary radioactive vitamin B_1 excretion in normal subjects patients with pernicious anemia and patients with malabsorption syndrome (sprue syndrome) as determined by Ellenbogen and Williams (personal communication) for the modification of the Schilling test described above [4 0 18] In general terms normal 48 hour values are never below 5 per cent of the 2 μg oral dose and values below 3 per cent invariably indicate defective absorption Tests giving results between 3 per cent and 5 per cent should be repeated

As a general rule in pernicious anemia the excretion of radioactive vitamin B_1 should be increased at least fivefold by the addition of intrinsic factor In malabsorption syndrome the addition of intrinsic factor may occasionally increase the excretion of vitamin B_1 as much as twofold (e g excretion over 48 hours may be 2 per cent with B_1 alone and 4 per cent with added intrinsic factor)

Recent work [3 140] raises the question as to whether it is of

TABLE 10 —*Average Urinary Excretion of an Oral Dose of μg of Radioactive Vitamin B Measured as Per Cent of the Oral Dose*

Day	Oral Treatment	Normal	Pernicious Anemia	Sprue
1	Vitamin B alone	10 5 (64)	0 (174)	0 7 (7)
2		4 4 (4)	0 3 (100)	0 3 (6)
3	B +Intrinsic Factor	12 1 (93)	(1 8)	0 7 (8)
4		3 (0)	3 3 (117)	0 4 (8)

Figures in parentheses represent the number of patients

The author is indebted to Drs L Ellenbogen and W L Williams for TABLES 9 and 10

tire 24 hour urine collection, measurement of the total volume and transfer of a 500 ml aliquot to a 1 or 2 liter Erlenmeyer flask. Twenty five ml of concentrated nitric acid are added to the aliquot and it is heated to boiling with the addition of boiling chips as needed. The nitric acid appears to inactivate the components of the urine responsible for foaming, thus allowing the urine to boil vigorously on a hot plate without foaming over. Boiling is continued until the total volume is less than 50 ml, at which time it is transferred to a suitable container and made up to 50 ml with tap water. This volume is suitable for counting on a flat scintillation crystal, the most inexpensive type available.

If the amount of radioactivity excreted in 18 hours is below the range of normal, careful inquiry is made to exclude a false low invalid result caused by loss of the radioactive vitamin B_{12} orally (vomiting), rectally (diarrhea), or by incomplete urine collection. A valid low result means the patient is absorbing an inadequate quantity of vitamin B_{12} because of either gastric or intestinal disorder, and repetition of the entire 48 hour procedure is mandatory, with the patient this time ingesting a standard dose of hog (or human) intrinsic factor concentrate with the oral dose of radioactive vitamin B_{12}.

This second test period allows division of patients unable to absorb vitamin B_{12} into two groups: those whose absorption of vitamin B_{12} is corrected to normal by intrinsic factor, and those whose absorption is not so corrected. The former group includes all patients with inadequate or absent secretion of intrinsic factor as the cause of their megaloblastic anemia; the latter includes those with vitamin B_{12} deficiency due to small intestinal disorder. The former group also includes some patients with fish tapeworm; in this special situation the worms may compete successfully for vitamin B_{12} with the host's intrinsic factor supply but may not be successful when the host's intrinsic factor is aided by an exogenous supply (especially when the radioactive vitamin is bound to the exogenous intrinsic factor before it reaches the site of localization of the tapeworm).

8 *Therapy*

PROPER TREATMENT of any of the megaloblastic anemias follows accurate differential diagnosis Since nearly all of the megalo blastic anemias are due to deficiency of vitamin B_1 folic acid or both either or both of these agents provide specific therapy in most cases

In the absence of complications such as concomitant iron deficiency or another disorder affecting the hematopoietic sys tem adequate specific therapy is followed by a dramatically rapid hematopoietic response[13] Within a maximum period of about eight hours after the start of therapy the megaloblasts in the bone marrow begin to be replaced by normoblasts This process is almost complete within two days and is nearly always complete within about a week

In the peripheral blood morphologic return to normal is much slower The Arneth count shift to the right gradually returns to normal over a period of one or two months and the macroovalocytes usually disappear over a period of one to three months

The rapid generation of erythrocytes results in an outpouring of reticulocytes into the peripheral blood with the maximal reticulocytosis being observed about the fourth to the twelfth day after the start of treatment The larger the therapeutic dose the earlier in this period is the maximal reticulocytosis usually observed By the same token the lower the patient s circulating erythrocyte count at the start of specific therapy the greater will be the relative number of reticulocytes to mature erythrocytes in the peripheral blood (i e per cent reticulocytosis) with therapy

The rate of rise of the red cell count and the packed cell volume (hematocrit) is greatest immediately following the peak rise in the reticulocyte count The rapidity of increase in eryth rocytes then tapers off and normal values are usually attained in approximately four to eight weeks after the start of treatment

clinical value to repeat the Schilling type test a third time using an oral dose of radioactive vitamin B_1 and simultaneous injection of 0 25 mg of carbamylcholine chloride (Carbachol) This third test would be reserved for those patients with inadequate secretion of intrinsic factor causing inadequate vitamin B_1 absorption correctible by addition of intrinsic factor Carbachol by stimulating gastric secretion of intrinsic factor would separate those patients *capable* of secreting an adequate quantity of intrinsic factor from those *not* capable of doing so

Such separation is of great value in helping to understand the cloudy etiology of the varying forms of pernicious anemia (see CHAPTER 4) and is an extremely desirable research undertaking In terms of clinical usefulness however such separation may not be of great value since regardless of whether or not the patient is *capable* of secreting intrinsic factor under intensive stimulus with Carbachol if such secretion does not occur *in physiologic circumstances* the patient will develop megaloblastic anemia due to vitamin B_1 deficiency This is well illustrated in a case report [138]

For this reason plus the fact that Carbachol is a very powerful parasympathomimetic agent which may be used only with great care in patients who are seriously ill [48] Carbachol stimulation is not recommended for routine use in evaluation of patients with megaloblastic anemia After the anemia has been adequately treated providing the patient has no complicating disorder it would be of interest to determine the effect of Carbachol on vitamin B_1 absorption

It must be kept in mind constantly that while the Schilling test is used as a measure of vitamin B_1 absorption it in fact measures the end product of the processes of absorption transport retention and renal excretion of the vitamin Anything affecting any of these four major variables will affect the test result Thus before concluding that absorption of vitamin B_{12} has been measured it must be ascertained that there are no abnormalities of the other three variables

Very slow conversion from a megaloblastic to a normoblastic marrow frequently occurs in patients with malabsorption syndrome even when they are treated with both vitamin B_{12} and folic acid Associated deficiencies of protein pyridoxine iron and other substances involved in hematopoiesis undoubtedly play a role in this slow response

Use of either vitamin B_{12} or folic acid alone therapeutically without determination of the etiology of the megaloblastic anemia cannot be condemned too strongly Folic acid administration will often produce temporary hematologic remission with eventual relapse in the patient who is vitamin B_{12}-deficient presumably through mobilization of the dwindling body stores of vitamin B_{12}[71] However it has been repeatedly demonstrated that this procedure allows the development and progression of neurologic damage[1] [7, 30, 3]

Indeed it has been suggested that folic acid administration may accelerate the speed of development of neurologic damage in the vitamin B_{12} deficient patient Both vitamin B_{12} and folic acid are involved in the synthesis of nucleic acids for blood formation[63] (see CHAPTER 3) but only vitamin B_{12} is required to maintain the integrity of the nervous system[4] (see CHAPTER 6) Administration of folic acid to the vitamin B_{12} deficient patient produces a drop in the already low serum vitamin B_{12} level which is proportional to the degree of the hematologic response[34] and thereby presumably deprives the nervous system still further by drawing the body s small remaining vitamin B_{12} reserves into blood formation at the expense of the nervous system[71]

In view of these facts shotgun administration of multivitamin preparations and hematinics has rightly received strong condemnation[13] Such preparations usually contain sufficient folic acid to support hematopoiesis and thereby mask the diagnosis of pernicious anemia while allowing neurologic damage due to vitamin B_{12} deficiency to progress Even as little as 0 3 mg of folic acid an amount which will seldom support hematologic function in pernicious anemia[3] may support such

When circumstances allow the hematologic response *to ther* apy should be documented by observing the *erythrocyte leuko cyte platelet* and reticulocyte counts the hematocrit and *hemoglobin* and the appearance of the peripheral blood smear *These* observations should be made just prior to the start of therapy and at weekly intervals thereafter for the first month then every two weeks during the second month In addition the reticulocyte count should be determined every two days from the start of treatment until the reticulocyte peak has been passed

Determination of the serum iron level has proved valuable in quickly assessing the effectiveness of vitamin B_1 or folic acid therapy [3, 4] Characteristically the serum iron level is elevated in untreated megaloblastic anemia regardless of whether it is due to deficiency of vitamin B_1 or of folic acid Within 24 hours after administration of the appropriate agent there is nearly always an abrupt and striking fall of the serum iron this is observed in practically all cases by 18 hours if the therapy is adequate *Values tend to fall to about 50 to 60 μ_g* of iron per 100 *ml of serum* within the 48 hour period and remain at that level until the blood count returns to normal If the therapeutic dosage is too small the serum iron tends to rise shortly after it has fallen If overt iron deficiency is impending the serum iron falls to values below 40 μg 100 ml with a range from 11 to 39 observed in 9 cases [4]

In this connection it should be noted that the presence of a normal serum iron level or the presence of iron in the bone marrow of a patient with *untreated* megaloblastic anemia does not necessarily indicate adequate iron stores for maintenance of normal hematopoiesis Generalized siderosis is frequent in megaloblastic anemia even among peoples consuming iron deficient diets [409] Much of this iron may be derived from disruption of intracellular iron containing enzymes and not from hemoglobin [409] Therefore only if storage iron is noted in the bone marrow *after* specific treatment of megaloblastic anemia can associated iron deficiency be ruled out

Very slow conversion from a megaloblastic to a normoblastic marrow frequently occurs in patients with malabsorption syndrome even when they are treated with both vitamin B_{12} and folic acid Associated deficiencies of protein pyridoxine iron and other substances involved in hematopoiesis undoubtedly play a role in this slow response

Use of either vitamin B_{12} or folic acid alone therapeutically without determination of the etiology of the megaloblastic anemia *cannot* be condemned too strongly Folic acid administration will often produce temporary hematologic remission with eventual relapse in the patient who is vitamin B_{12} deficient presumably through mobilization of the dwindling body stores of vitamin B_{12} [1, … 9] However it has been repeatedly demonstrated that this procedure allows the development and progression of neurologic damage [1, … 7, 30–…]

Indeed it has been suggested that folic acid administration may accelerate the speed of development of neurologic damage in the vitamin B_{12} deficient patient Both vitamin B_{12} and folic acid are involved in the synthesis of nucleic acids for blood formation [33] (see CHAPTER 3) but only vitamin B_{12} is required to maintain the integrity of the nervous system [4, 3] (see CHAPTER 6) Administration of folic acid to the vitamin B_{12} deficient patient produces a drop in the already low serum vitamin B_{12} level which is proportional to the degree of the hematologic response [34] and thereby presumably deprives the nervous system still further by drawing the body s small remaining vitamin B_{12} reserves into blood formation at the expense of the nervous system [71]

In view of these facts shotgun administration of multivitamin preparations and hematinics has rightly received strong condemnation [13] Such preparations usually contain sufficient folic acid to support hematopoiesis and thereby mask the diagnosis of pernicious anemia while allowing neurologic damage due to vitamin B_{12} deficiency to progress Even as little as 0 3 mg of folic acid an amount which will seldom support hematologic function in pernicious anemia [25] may support such

function when supplemented by the amount of folic acid in the patient s diet

Particularly pernicious is the uncontrolled widespread public promotion and sale of geriatric vitamin preparations containing folic acid [30] The sales campaigns for these preparations are aimed directly at the elderly part of the population in which nearly all cases of pernicious anemia occur The hapless victims of these campaigns are those individuals with vitamin B_{12} deficiency due to lack of intrinsic factor who purchase with their geriatric vitamins an excellent chance for irreversible neurologic damage Such sales are not controlled by law and the promoters will not remove folic acid from their preparations for fear of not being able to compete with other *complete* vitamin supplements

Because of the seriousness of the neurologic damage which results from prolonged vitamin B_{12} deprivation when the condition of the patient makes therapy necessary prior to establishment of the etiology of the megaloblastic anemia vitamin B_{12} should be the initial therapeutic agent [531] and should be given by injection

In the folic acid deficient patient treatment with small doses of vitamin B_{12} may aggravate the clinical manifestations of the folic acid lack [63] Severe painful glossitis atrophy of the lingual papillae and cheilosis may develop or grow worse It is not known whether the same phenomena may occur when large doses of vitamin B_{12} are used but presumably they would

In general terms temporary hematologic remission in the vitamin B_{12} deficient patient is more frequently accomplished with folic acid than is such remission in the folic acid deprived patient when vitamin B_{12} is administered Most B_{12} deficient patients will sustain a temporary hematologic remission the first time they are given folic acid The period of remission becomes shorter with each subsequent course of folic acid until eventually the patient becomes totally refractory to this agent with development of a hypocellular nonmegaloblastic bone marrow

and subsequently only a slow response to vitamin B_{12} *unassociated with a reticulocyte response* [1, 418]

Folic acid is unnecessary and unwarranted in the treatment of pure vitamin B_{12} deficiency states such as that associated with pernicious anemia It is true however that folic acid metabolism is interfered with in vitamin B_{12} deficiency For example folinic acid rather than folic acid is normally stored in the liver [34, 39, 9] In vitamin B_{12} deficiency however a sizable portion of the liver store consists of folic acid [34] This suggests that the ability to convert folic acid to folinic acid and possibly to convert folic acid into any metabolically useful form may depend in large part on the availability of vitamin B_{12}

Subnormal folic acid levels have been found in the *whole blood* of some patients with pernicious anemia in relapse [4, 3] but *serum* folic acid levels have been normal in all such cases [489] Other findings suggestive of possible folic acid deficiency in patients with pernicious anemia have included reduction in urinary excretion of folic acid before and shortly after vitamin B_{12} therapy lowering of the blood folic acid levels following vitamin B_{12} injections and rapid clearance of intravenously injected folic acid in some patients with untreated pernicious anemia [314, 38]

In their review of folic acid and vitamin B_{12} interrelationships in pernicious anemia supplemented by 12 years of their own studies Will and his associates [71] point out that the various findings suggestive of associated folic acid deficiency in pernicious anemia are *susceptible of other interpretations* primarily in terms of interference in folic acid metabolism due to lack of vitamin B_{12} They observe that folic and folinic acid in serum may be used more rapidly in hematopoiesis with tissue stores being used later and that tissue depots may more avidly take up and retain folic and folinic acid when the vitamin B_{12} supply is inadequate

Thus while there is at present no direct evidence of inadequate stores of folic acid (although folinic acid stores may be depleted) in vitamin B_{12} deficiency states [34] normal folic acid

metabolism does appear to be dependent on an adequate supply of vitamin B_1 which may be involved in release of folinic acid from its tissue conjugates facilitation of the reduction of folic acid to folinic acid and in other biochemical mechanisms promoting the formation of N^{10} formyl tetrahydrofolic acid [71] currently believed to be the major active folic acid metabolite [66]

In summary there is probably no folic acid deficiency generally in patients with pernicious anemia in terms of *supply* of folic acid but there is probably a folic acid deficiency in terms of *impaired utilization* of folic acid due to lack of vitamin B_1 Similar impaired utilization of folic acid probably occurs in all of the vitamin B_1 deficiency states The degree of impaired folic acid utilization is probably proportional to the degree of vitamin B_1 deficiency

Hematologic response of the folic acid deficient patient to vitamin B_1 administration is frequently incomplete and may not occur at all Almost every hematologist has had the experience of giving as much as 0 1 to 1 mg of vitamin B_1 to a patient with megaloblastic anemia and observing that the marrow remained megaloblastic Such cases almost invariably turn out to be caused by folic acid deficiency and respond dramatically to administration of this agent

I VITAMIN B_1

A Parenteral

The only uniformly reliable way to administer vitamin B_1 is by intramuscular or deep subcutaneous injection The vitamin B_1 deficient patient requires sufficient vitamin B_1 by this route of administration both to produce remission and to build up the depleted body stores of this vitamin [34–36, 539] In this connection it is an interesting fact that not all of the liver stores of vitamin B_1 are available for blood formation Patients with pernicious anemia may sometimes be noted to have a sponta neous remission if they develop hepatitis [463, 464] which releases

the liver vitamin B_{12} *stores into the blood stream*[46, 540] *It must* be remembered that vitamin B_1 is required wherever nucleo protein synthesis is taking place and this involves almost every tissue in the body not only the hematopoietic and nervous systems

A great deal of research has been devoted to an attempt to establish an ideal treatment schedule for the vitamin B_1 defi ciency states caused by inability to absorb the vitamin[541–546] This work has shown that 90 to 95 per cent of an initial injected dose up to 50 μg is retained but the percentage retained of doses over this amount falls rapidly at a rate that is different for each patient

Thus within 48 hours after injections of 100 to 1000 μg of vitamin B_1 from 50 to 98 per cent of the injected dose may appear in the urine[543] although many patients may retain as much as 40 per cent of an initial 1000 μg dose[4]

A single injection of 20 μg of vitamin B_1 will raise the serum level of a pernicious anemia patient to normal for about one to three weeks The period for which serum B_1 levels remain normal increases by smaller and smaller increments as the quan tity of vitamin injected is increased so that an injection of 160 μg may keep the serum B_1 normal for about two to seven weeks and an injection of as much as 1000 μg for only about five to eight weeks[46]

These studies all indicated that the capacity of the serum and tissues to absorb vitamin B_1 is limited to a certain maximum per unit time and excess amounts of vitamin over this capacity are rapidly excreted In addition patients who lack intrinsic factor may have a reduced serum vitamin B_1 binding capa city[47] although this has not yet been firmly established This may be related to a possibly inadequate mechanism for storage of vitamin B_1 which has not yet been demonstrated[109, 06]

However there are good arguments nonetheless in favor of initial therapy with large doses of vitamin B_1 and of mainte nance with similar doses (Vitamin B_1 is nontoxic even when given in single doses as high as 3000 μg[48, 549]) The vitamin B_1

deficient patient achieves a normal serum B_{12} level with a single injection of less than 300 μg but this level is in the low part of the normal range. Genuinely normal levels in the range of the normal mean are only achieved (after a single injection) when the amount injected is in excess of 300 μg.[46]

Resaturation of the body stores of vitamin B_{12} takes place more rapidly when large doses are given.[46] Recent studies suggest that even with such doses resaturation is difficult to achieve within a three week period.[4]

Should future studies demonstrate that vitamin B_{12} is stored in the human liver as a B_{12} co-enzyme as it may be in rabbit liver,[41a] such a B_{12} co-enzyme may prove to saturate the body stores more readily.

It has also been noted that a significant build-up in the vitamin B_{12} content of the spinal fluid can be obtained rapidly with daily injections of 1000 μg of vitamin B_{12}.[43] This may be of significance in recovery from neurologic damage and in aborting further such damage, especially if, in contrast to other tissues, the brain takes up B_{12} more slowly but shows a steady increment in the amount stored with continued injections.[9] In cases with neurologic involvement, it is not yet clear whether continuous administration of daily large doses of vitamin B_{12} is really more valuable than administration of moderate doses. Evaluation is complicated by difficulty in determining how much improvement is owing to the treatment itself and how much is owing to the frequent visits to the patient and testing of his dexterity and concern as to his mood.[51]

While 1 μg/day has been generally accepted as the minimal maintenance requirement for vitamin B_{12}, approximately 6 μg/day may prove a better estimate for optimal well-being of the patient.

Keeping all of the above in mind, the most rational approach to therapy with vitamin B_{12} at the present time in the private practice of medicine is the administration of 1000 μg of vitamin B_{12} (in 1 ml. of solution) every other day for a total of 5 doses as initial treatment to replenish substantially the body

stores followed by injection of 500 to 1000 μg /month as a main tenance dosage The initial injections should be given every other day rather than every day because the percentage retention of each dose is substantially reduced if it is given only 24 hours after a prior dose[541 546]

When there is neurologic damage *initial treatment should* probably include 10 to 15 doses of 1000 μg at daily intervals followed by 1000 μg once or twice weekly for several months then gradual spacing of the interval between doses ending with 1000 μg at monthly intervals after a period in excess of a year when it appears no more neurologic recovery is likely to occur [b]

At some future date a reliable depot vitamin B_1 preparation may be developed which will allow a longer interval between maintenance doses An insoluble complex of vitamin B_1 zinc and tannic acid is now being studied as such a repository prepa ration Early reports mention no undesirable local or systemic effects due to the zinc or tannate [3 5 3] Further evaluation is required to be certain such effects do not occur and that the slowly released material is in fact vitamin B_1 in a form avail able for human metabolism

It is true that most patients can be maintained on monthly injections of vitamin B_1 as low as 50 μg[5c] or 100 μg [4] but this may not always be adequate to maintain the body stores and to maintain the patient in hematologic and neurologic remission in the face of any increased metabolic requirement or decreased capacity to respond to vitamin B_1 such as accompanies illness (A possible case in point [1] has been alluded to in the section Inability to Utilize Vitamin B_1)

Maintenance therapy must continue for the lifetime of the patient who lacks intrinsic factor This must be forcefully ex plained to the patient to prevent his forgetting all about it after the first injections of vitamin B_1 produce a typical drama tic subjective and objective improvement The patient who as sumes after the first injections that he is cured often winds up in relapse with irreversible neurologic damage

deficient patient achieves a normal serum B_1 level with a single injection of less than 300 μg but this level is in the low part of the normal range Genuinely normal levels in the range of the normal mean are only achieved (after a single injection) when the amount injected is in excess of 300 μg [46]

Resaturation of the body stores of vitamin B_1 takes place more rapidly when large doses are given [46] Recent studies suggest that even with such doses resaturation is difficult to achieve within a three week period [4]

Should future studies demonstrate that vitamin B_1 is stored in the human liver as a B_1 co enzyme as it may be in rabbit liver [41?] such a B_1 co enzyme may prove to saturate the body stores more readily

It has also been noted that a significant build up in the vitamin B_1 content of the spinal fluid can be obtained rapidly with daily injections of 1000 μg of vitamin B_1 [43] This may be of significance in recovery from neurologic damage and in aborting further such damage especially if in contrast to other tissues the brain takes up B_1 more slowly but shows a steady increment in the amount stored with continued injections [0] In cases with neurologic involvement it is not yet clear whether continuous administration of daily large doses of vitamin B_1 is really more valuable than administration of moderate doses Evaluation is complicated by difficulty in determining how much improvement is owing to the treatment itself and how much is owing to the frequent visits to the patient and testing of his dexterity and concern as to his mood [51]

While 1 μg/day has been generally accepted as the minimal maintenance requirement for vitamin B_1 approximately 6 μg/day may prove a better estimate for optimal well being of the patient

Keeping all of the above in mind the most rational approach to therapy with vitamin B_1 at the present time in the private practice of medicine is the administration of 1000 μg of vitamin B_1 (in 1 ml of solution) every other day for a total of 5 doses as initial treatment to replenish substantially the body

ciency This should include thorough monthly examination of a peripheral blood smear for macroovalocytosis and an increase in the number of hypersegmented polymorphonuclear leuko cytes and complete neurologic examination Where facilities for serum vitamin B_1 determination are available this should also be obtained at bimonthly intervals

1 Vitamin B_1 Alone

The absorption of physiologic (0 5 to 2 μg) amounts of vita min B_{12} is primarily dependent on the presence of intrinsic fac tor and a functionally normal small intestine In a patient who lacks intrinsic factor and has megaloblastic anemia any remis sion occurring while ingesting such doses (obtainable in a good diet) falls in the category of a spontaneous remission[76 60 5 6] perhaps related in part to folic acid in the diet

Oral maintenance therapy with physiologic doses of vitamin B_1 is reserved exclusively for patients whose vitamin B_1 defi ciency was the result solely of inadequate ingestion of the vita min or a corrected inadequate absorption uncomplicated by defective gastric secretion of intrinsic factor or malabsorption of vitamin B_1

The absorption of supraphysiologic amounts of vitamin B_1 is not intrinsic factor dependent and appears to take place by a mass action effect possibly diffusion[93 9 11 5 9 60] About 1 5 μg of a 50 μg dose is absorbed mainly through this mechanism and about 15 μg of a 1000 μg dose[5 7]

Thus some patients whose vitamin B_1 deficiency was caused by inadequate or absent secretion of intrinsic factor and who have no small intestinal disorder may be maintained on small oral doses of as little as 15 to 50 μg daily [8] though relapse is frequent [8] Probably most patients may be marginally main tained by daily doses in excess of 100 μg[96 5 9 560] Weekly single oral doses of 1000 μg have been successful in maintaining a large group of such patients but their serum vitamin B_1 levels tend to be suboptimal as do the serum levels of patients taking 50 μg three times daily[60] Therefore oral maintenance with

Because of the increased incidence of gastric cancer in patients with pernicious anemia, it may be wise to evaluate the patient at yearly intervals for a possible early gastric malignancy. Such evaluation may include roentgen studies as well as gastroscopy and microscopic study of gastric cells obtained by aspiration or by abrasive techniques.[5] It should certainly include thorough questioning of the patient for any symptoms of possible gastric origin. Such questioning is best done informally and without undue emphasis to avoid cancerophobia.

Those few cases in which absorptive defect for vitamin B_{12} is correctible by surgery (structural abnormalities of the small intestine) may cease receiving maintenance therapy after such surgical correction and after demonstration that the ability to absorb vitamin B_{12} has been made normal is confirmed by a Schilling test or another test of B_{12} absorption.

Patients whose vitamin B_{12} deficiency was due to dietary inadequacy or fish tapeworm may have their maintenance therapy terminated when their ingestion of vitamin B_{12} is adequate and anthelminthic therapy has expelled the worms. Before such termination it should be determined by a Schilling test or other study that during the period of vitamin B_{12} deprivation the patient did not lose the ability to produce intrinsic factor because of gastric atrophy resulting from the vitamin deficiency.

B. Oral

Initiation of treatment for vitamin B_{12} deficiency should always be with parenteral administration of the vitamin. In the best circumstances, even in a normal gastrointestinal tract, absorption of vitamin B_{12} is extremely limited. It has not yet been demonstrated that it is possible to fill the depleted body stores of the B_{12} deficient patient using any oral preparation.

Once repletion of the body stores has been accomplished by parenteral administration of vitamin B_{12}, one of the various oral preparations may be used for maintenance in certain selected patients *provided the patients are carefully checked at regular intervals for any evidence of recurrent vitamin B_{12} defi-*

hog intrinsic factor concentrate is due to development of resistance to some component of the concentrate. The absorption block may be partially or completely overcome by very large doses of concentrate.[9, 11, 4] Normal human gastric juice will still enhance vitamin B_{12} absorption after partially purified hog intrinsic factor concentrates fail.[16, 9] For some reason relatively unpurified preparations of hog stomach and pyloric mucosa have not been associated with the development of resistance.[9]

Antibody to some component of hog intrinsic factor concentrates may develop in the blood of patients on prolonged oral vitamin B_{12} intrinsic factor concentrate therapy,[f] but no relation has been demonstrated between the presence of such antibody and refractoriness to such oral treatment. It has not yet been determined whether this antibody is to intrinsic factor itself or to a contaminant in the concentrates. The antibody appears in vitro to precipitate human intrinsic factor concentrate as well as that from hogs,[f] but whether the specific precipitate is intrinsic factor or another substance to which intrinsic factor loosely attaches has not been determined. A possible culprit may be blood group A substance, which is present in the most highly purified hog intrinsic factor concentrate currently available,[7] just as it is found in less purified concentrates.[8, 81] Rabbits immunized to hog intrinsic factor concentrate[81] (or to A substance) do not produce anti-A agglutinins.

As has been suggested,[6] it is possible that a local antibody reaction to some component of heterologous intrinsic factor concentrate may develop at the hypothetical intestinal receptor site for intrinsic factor,[111, 11] resulting in rejection of such intrinsic factor.

At the present time, maintenance therapy with oral vitamin B_{12} hog intrinsic factor concentrates (or concentrates from other animal species than man) should be used only with constant extreme vigilance against the development of refractoriness, including serial estimation of the vitamin B_{12} levels of serum.[f3, f] It is possible that pure intrinsic factor, when isolated, will be an

vitamin B_1 at weekly intervals requires administration of more than 1000 μg each week [61]

In general oral treatment should not be employed if small intestinal dysfunction exists

Oral treatment with vitamin B_1 alone may be effective when the patient is very diligent in taking every dose of medication and returns at regular intervals no more than a month apart for thorough evaluation of his hematologic neurologic and general clinical status preferably with determination of his serum vitamin B_1 level as well However oral therapy with vitamin B_1 even in the best of circumstances is not as reliable as parenteral therapy and cannot be recommended

2 Vitamin B_1 Plus Hog Intrinsic Factor Concentrate

A priori it would appear that ideal therapy for the patient who lacks intrinsic factor would be oral administration of the substance specifically lacking This is especially true if intrinsic factor proves to be involved in vitamin B_1 transport and storage as may possibly be the case [10, 10, 108, 109, 0F, 4, 03, 04] It is of great interest in this connection that the most active vitamin B_1 containing enzyme fraction of rat liver precipitates in 40 to 60 per cent ammonium sulfate [43] which is almost identical to the 35 to 55 per cent ammonium sulfate concentration in which intrinsic factor precipitates [119]

Concentrates of intrinsic factor derived from hog gastric pyloric and duodenal mucosa have been used successfully to maintain patients who lack intrinsic factor [51, 67] However an increasing number of cases have been reported in which maintenance therapy with some oral vitamin B_1 partially purified hog intrinsic factor combinations has been associated with development of inability of the patient to absorb the combined product and eventual failure of such treatment [60] This phenomenon has not occurred to a great extent in two series of patients however [5, 73]

The evidence so far suggests that long term failure of oral therapy with heterologous preparations of partially purified

whose baseline secretion of intrinsic factor is low but in whom administration of a secretagogue stimulates normal intrinsic factor output [141, 88]

There are two immediately obvious mechanisms whereby an added agent may enhance vitamin B_{12} absorption in a normal subject (1) by stimulating gastric secretion of intrinsic factor and (2) by combining with vitamin B_{12} in a bond which is only broken in the alkaline medium of the small intestine The latter mechanism is effective because for unknown reasons vitamin B_{12} which bypasses the stomach is better absorbed than vitamin B_{12} which passes through the stomach in uncombined form [3, 4, 5, 6] Other mechanisms may exist but they are as yet undefined

The only way the practicing physician can protect his patients from useless oral therapies is to ignore the pharmaceutical firm advertisements that flood his mail and his medical journals and rely on treatment that he knows has stood the test of time The physician should realize that just because many of these oral therapies are promoted only on an ethical basis i e only to physicians such promotion is no guarantee of efficacy

Other than for their content of vitamin B_{12} alone oral therapy with vitamin B_{12} and the various absorption enhancing agents has no place in the treatment of megaloblastic anemias at the present time

II FOLIC ACID

Folic acid in oral doses of 5 to 10 mg daily is effective in the treatment of most cases of folic acid deficiency [9, 93] An occasional refractory patient may require a larger dose [56] but such patients may suffer from multiple deficiencies and require correction of such deficiencies rather than larger doses of folic acid Cases in point are those with ascorbic acid deficiency

It is noteworthy that even in those patients whose folic acid deficiency is due to impaired absorption of the vitamin oral therapy with 5 to 10 mg daily doses is effective Such doses are probably considerably in excess of the minimal daily requirement for this vitamin and may pass whatever intestinal barrier

oral therapeutic agent of choice At present such treatment can not be recommended The most recent work available[11] [51b] suggests species specificity may be a problem with pure intrinsic factor

3 Other Oral Therapies with Vitamin B_1 and Added Agents

From time to time a claim appears in the literature that vitamin B_1 combined with one agent or another is or may be effective oral therapy for pernicious anemia [6] [83] Such improved oral therapies usually turn out to depend for their effect primarily on their content of vitamin B_1 and offer no significant advantage over oral treatment with vitamin B_1 alone [9] [84–86]

A serious problem for the practicing physician as well as the public at large is the tremendous promotional campaign given almost every few months by many pharmaceutical firms to various combinations of vitamin B_1 and any one of a number of agents which are said to enhance markedly vitamin B_1 absorption Any one of literally hundreds of agents taken orally may enhance absorption of vitamin B_1 *by normal individuals* but only intrinsic factor will generally enhance the absorption of vitamin B_1 in the patient who lacks this agent ACTH steroids or calcium in some cases may improve intestinal absorption of vitamin B_1 in patients with the malabsorption syndrome The advertisements in medical journals of oral vitamin B_1 combinations with this or that miraculous absorption enhancement factor adroitly omit any mention of the basic fact that the miraculous absorption enhancement factor may only work when the patient does not need treatment with vitamin B_1 in the first place

One such agent d sorbitol is a hexahydric alcohol which may enhance the absorption of vitamin B_1 in normal individuals [87] and in persons with normal baseline ability to absorb vitamin B_1 [88] but not in patients unable to secrete intrinsic factor [89] If it has any effect at all which has been questioned [90] it may act by stimulating gastric secretion of intrinsic factor [91] and may be of use in a single very limited situation that of the patient

While they also compete with citrovorum factor which is formed in the body from folic acid and convertible to the same active form as is folic acid [16] they do not block its utilization [30, 31, 93]

IV LIVER EXTRACT

It is unlikely that liver extract has any therapeutic usefulness not due to its content of vitamin B_1 and folic acid Liver extract has many disadvantages as a therapeutic agent when compared to the pure vitamins it is more costly less concentrated more likely to produce pain at injection sites and may produce allergic reactions [86, 97]

While it is possible that another as yet not isolated factor may be present in liver extract that may be effective in a rare case of megaloblastic anemia unresponsive to vitamin B_1 or folic acid [98] no such factor has yet been defined

It has been claimed [599, 600] that there is a protein synthesis factor present in liver extracts which is neither vitamin B_1 nor folic acid The claim was based on two observations neither of which has been confirmed by most other observers First it was stated that reduced prothrombin times generally occur in patients with pernicious anemia and related macrocytic anemias and that liver extracts more rapidly restore such reduced prothrombin times to normal than either vitamin B_1 or folic acid Second it was stated that liver extract restored macrocytosis to normocytosis more efficiently than vitamin B_1 or folic acid

It is possible that in pernicious anemia liver stores of agents other than vitamin B_1 and folic acid are disturbed There is usually an irregular pattern of liver dysfunction in pernicious anemia [462] As to the possible need for liver extract to restore prothrombin time to normal reduction in prothrombin time sometimes occurs in association with pernicious anemia [6 1] but this phenomenon may well be the exception rather than the rule [384] It may be related to the anorexia nausea and vomiting of untreated patients [60] When it occurs it is restored to normal after adequate specific therapy with vitamin B_1 alone The

may exist to folic acid absorption by a mass action effect It is also possible that unconjugated folic acid is absorbed with relative ease whereas the folic acid conjugates present in food may be absorbed with greater difficulty or may have to be deconjugated prior to absorption

It is of interest that patients with idiopathic steatorrhea who have been on adrenal steroid therapy for many years may have persistently low serum folic acid levels with macrocytosis and an Arneth count shift to the right despite ingestion of 0 25 mg of folic acid three times daily This suggests that the persistence of jejunal mucosal damage is accompanied by persistence of inability to absorb physiologic doses of folic acid (i e doses in the range of 0 5 mg or less)

Parenteral administration of folic acid may be required in the presence of severe diarrhea and in megaloblastic anemia in infancy if there is difficulty in giving the vitamin perorally Generally the dose given parenterally is as large as that given by mouth but doses of 1 mg of folic acid are adequate when injected to produce remission Repletion of body stores of folic acid may require larger doses but these may usually be given subsequently by mouth

Maintenance therapy after repletion of the body stores may be carried out with as little as 0 25 to 0 5 mg of folic acid daily and a good diet The latter alone is generally effective in supplying an adequate amount of folic acid In severe malabsorption syndromes however 5 to 10 mg daily by mouth may be required because of persistent inability to absorb physiologic doses

III CITROVORUM FACTOR (FOLINIC ACID)

The one specific indication for citrovorum factor therapy in doses of 3 to 6 mg is to overcome the effects of the folic acid antagonists employed in cancer chemotherapy [4] These agents such as Aminopterin both compete with and irreversibly block the conversion of folic acid to its metabolically useful form for periods of two to three weeks after administration [30 9]

Various workers have suggested an interrelationship between ascorbic acid and cyanocobalamin because untreated patients with megaloblastic anemia often have low serum ascorbic acid levels and intravenously administered ascorbic acid disappears more rapidly in the vitamin B_1 deficient patient than it does in the normal subject [608, 609] However the nature of the association of B_{12} deficiency and the abnormality of ascorbic acid metabolism is by no means clear though it is evident that the latter plays no fundamental part in the production of a megaloblastic anemia in B_1 deficiency [608] Treatment of nearly all megaloblastic anemias except perhaps some of the megaloblastic anemias of infancy and the rare cases that may occur with scurvy does not require the use of ascorbic acid nor does such use have any known value

In this conection it should be noted that the finding of *any ascorbic acid at all* in plasma or whole blood is incompatible with a diagnosis of scurvy unless the vitamin has recently been administered [81] A level up to 4 mg per cent in the buffy coat of the blood is compatible with scurvy however [60]

VI BLOOD TRANSFUSION

When the initial hematocrit is below 15 per cent particularly in elderly persons a single transfusion preferably of packed red cells is advisable to sustain the patient until response to vitamin B_1 (or folic acid) can occur [86] In the patient with dehydration associated with the malabsorption syndrome whole blood transfusion is preferable unless congestive heart failure is present In the absence of a very low hematocrit or serious complicating factors such as infections or cardiac disorders it is rarely necessary to transfuse patients with megaloblastic anemia since the response to specific therapy is rapid enough to restore the hematologic status to normal in a short time

It must be emphasized that even with a hematocrit below 15 per cent or a hemoglobin below 5 Gm /100 ml of blood more than one transfusion is rarely either needed or desirable It is

hemorrhagic manifestations sometimes seen in untreated pernicious anemia are in the author s experience uniformly associated with thrombocytopenia which clears on specific therapy and only irregularly associated with reduced prothrombin time

Perhaps the claim of a protein synthesis factor arose in part from inclusion of patients with the malabsorption syndrome Such patients frequently not only absorb vitamin B_1 and folic acid poorly thereby developing a megaloblastic anemia but also have grossly impaired absorption of vitamin K for synthesis of prothrombin and other serum factors involved in blood clotting[603,604] In these patients injection of vitamin K_1 rapidly restores prothrombin Factor VII and Stuart factor to normal[603] Here too no specific value of liver extract over adequate vitamin B_1 folic acid and vitamin K administration has been documented

There is no objective evidence for the claim that liver extracts restore macrocytosis to normocytosis more rapidly than equivalent amounts of vitamin B_1 and folic acid Treatment of the megaloblastic anemias with adequate specific therapy is generally efficient in eliminating all vestiges of the anemia including the macrocytosis Two to four months of therapy are usually required

V ASCORBIC ACID (Vitamin C)

The patient with megaloblastic anemia due at least in part to ascorbic acid deficiency should receive about 2 Gm of vitamin C by mouth to resaturate his tissues administered in divided doses of 100 mg five times a day[606,607] An equal amount may be given parenterally if the patient can not take oral medication Within 24 hours on this regimen any active bleeding ceases After this maintenance therapy consists of 200 mg per day until recovery is assured and 100 mg daily thereafter The above doses may be halved for infants[607] The maintenance requirement for ascorbic acid in infants is met by ingesting orange juice regularly and in children and adults by ingesting a well balanced diet including citrus fruits tomatoes and vegetables

the treatment of megaloblastic anemias other than those associated with generalized severe malabsorption

VIII OTHER THERAPY

Treatment other than as outlined above would depend on the condition causing the megaloblastic anemia Such treatment would include insuring an adequate diet in all cases and a gluten free diet in childhood and adult coeliac disease [19 4] prescribing anthelminthics in cases of fish tapeworm infestation appropriate antibiotics in the blind loop syndrome surgery where feasible to correct structural abnormalities of the small intestine and correction of endocrine disorders In addition appropriate supportive measures are self evident as is prompt and vigorous antibiotic therapy in old patients with pernicious anemia in relapse who develop pneumonia or urinary tract infections[536] The key to such treatment is accurate differential diagnosis of the cause of the megaloblastic anemia supplemented by *thorough evaluation of the patient for any related or* unrelated disorders

deplorable that so many unnecessarily panic stricken physicians give not one transfusion but so many that circulatory overload may result [31] the patient is unnecessarily endangered [610] and the hematologic response to therapy is masked For these physicians a few quotes from Crosby s editorial in the journal Blood on the misuse of blood transfusion [610] may be in order Anemia alone is not a sufficient justification for transfusion Most bed fast patients are comfortable with as little as 5 or 6 grams of hemoglobin The dangers of blood transfusion infection hemosiderosis and transfusion reaction are well known Thoughtless prescription of blood transfusion is playing Rus sian roulette with bottles of blood instead of a revolver

VII ADRENOCORTICOTROPHIC HORMONE (ACTH) AND ADRENOCORTICAL STEROIDS

ACTH or the adrenocortical steroids will often correct the absorptive defect for vitamin B_{12} associated with tropical sprue [9, 13, 14] and are useful in the management of that dis ease and often of other disorders presenting with the clinical picture of the malabsorption syndrome [11, 61] including regional ileitis [9] They did not correct the absorptive defect for vitamin B_{12} in nontropical sprue in most of the cases seen by the author

Hematologic response to adrenocortical steroid therapy has been observed in some cases of addisonian pernicious anemia and of adult coeliac disease in 2 cases of rheumatoid arthritis associated with megaloblastic anemia and a low serum vitamin B_{12} level despite normal vitamin B_{12} absorption and 1 case of megaloblastic anemia after partial gastrectomy [14, 1] In only 1 case a patient with addisonian pernicious anemia [1] was the absorption of vitamin B_{12} *enhanced during the steroid therapy* in that case intrinsic factor secretion did not appear to have been enhanced despite the hematologic response The mechanism of action of the steroid therapy in these few cases is unknown and the hematologic response is generally slow when it occurs at all For these reasons steroids are not considered to have a place in

References

1 EHRLICH P De und Regeneration roter Blut cheiben Verhandl deutsch Gesellsch d Char (June 10 and Dec 9) 1880

2 REISNER E H JR The nature and significance of megaloblastic blood formation Blood 13 313 1958

3 MOLLIN D L Radioactive vitamin B_{12} in the study of blood diseases Brit M Bull 15 8 1959

4 GIRDWOOD R H The role of folic acid in the blood disorders Brit M Bull 15 14 1959

5 WILLS L Pernicious anemia nutritional macrocytic anemia and tropical sprue a discussion Blood 3 36 1948

6 —— Studies in pernicious anemia of pregnancy VI Tropical macrocytic anemia as a deficiency disease with special reference to the vitamin B complex Indian J M Res 1 699 1934

7 FOY H KONDI A AND SALMA B The anemias of the tropics India and Ceylon J Trop Med 61 27 1958 Tr Roy Soc Trop Med & Hyg 52 46 1958

8 DAS GUPTA C R AND CHATTERJEA J B The role of synthetic folic acid (L casei factor) in the treatment of nutritional macrocytic anemia Ind M Gaz 81 402 1946

9 FAIRLEY N H BROMFIELD R J FOY H AND KONDI A Nutritional macrocytic anemia in Macedonia A preliminary report Tr Roy Trop Med & Hyg 32 132 1938

10 TASKER P W G MOLLIN D L AND BERRIMAN H Vitamin B deficiency in the megaloblastic anemias of Malaya Brit J Haemat 4 167 1958

11 TOTTERMAN G On the occurrence of pernicious tapeworm anemia in D phyllobothrium carriers Acta med scandinav 118 410 1944

12 FRIEDLANDER R D Racial factor in pernicious anemia Am J M Sc 187 634 1934

13 WINTROBE M M Clinical Hematology Philadelphia Lea & Febiger 1956

13a BINGHAM J The macrocytosis of hepatic disease I Thin macrocytosis Blood 14 694 1959

14 NORDENSON N G SEGERDAHL E STRANDELL B AND WALLMAN CARLSSON C Die Frequenz und geographische Verbreitung der perniziosen Anämie in Schweden Acta med scand nav 9 ? ? 1938

15 REISNER E H JR WOLFF J A MCKAY R J JR AND DOYLE E F Juvenile pernicious anemia Pediatrics 8 88 1951

16 EDITORIAL Age and vitamin B_{12} metabolism Nutrit Re 17 40 1959

17 KRISTENSEN H I O AND GORMSEN H Vitamin B_{12} deficiency in uncharacteristic macrocytic anemia Comparison of bone marrow findings and vitamin B levels in plasma Acta med scandinav 16? 415 1958

18 TAUBER S A GOODHART R S HSU J M BLUMBERG N KASSAB J AND CHOW B F Vitamin B deficiency in the aged Geriatrics 1 68 1957

Summary

The literature on the megaloblastic anemias has been reviewed with special emphasis on recent advances in knowledge and supplemented by the author's experiences and research with patients and in the laboratory. The megaloblastic anemias have been discussed in terms of their incidence, hematologic morphology, biochemical basis, etiologic classification, clinical picture, differential diagnosis, and therapy.

The heart of this monograph has been the presentation of the etiologic classification of the megaloblastic anemias. They are seen to be primarily due to inadequate ingestion, absorption, or utilization of vitamin B_{12} or folic acid. The possible causes in each case are presented.

Clear understanding of the possible causes of each of these inadequacies forms the basis for accurate differential diagnosis and, in turn, of proper and specific treatment of each case.

Rapid and accurate recognition, differential diagnosis, and treatment of a patient with a megaloblastic anemia frequently provide the richest reward in the practice of medicine: achievement of an almost overnight transformation from a moribund, seemingly hopeless state to one of vigor, health, and vitality.

39 SWENDSEID M E BETHELL F H AND ACKERMANN W W The intracellular distribution of vitam n B and folinic acid in mouse liver J Biol Che n *190* /91 1951
40 HERBERT V STORER J AND SLAET T H Unpublished data
41 UNDRITZ E Sandoz Atlas of Haematology Basle Frobenius 1952
42 WEICKER H Hamo Zytomorpholog e des B Mangels in vivo *In* Heinrich H C Vitamin B_{12} und Intr nsic Factor Stuttgart Ferdinand Enke 1957
43 FUDENBERG H AND ESTREN S Non addison an megaloblastic anemia The intermediate megaloblast in the differential diagnos s of pernicious an l related anemias Am J Med *5* 198 19 8
44 DACIE J V Discus ion Brit M J *1* 02 1948
45 EDITORIAL The intermediate megaloblast Lancet *1* 105 19 9
46 MOLLIN D L The intermediate megalobla t Br t M J *1* 302 1959
47 DACIE J V AND WHITE J C Erythropoies s with part cular reference to its study by bicpsy of human bone marro A rev ew J Clin Path ? 1 1949
48 BARBIER F Un cas particul er l hypersegm entation const tutionelle des noyaux des neutiophiles chez l homme Acta haemat *19* 121 1958
48a FEDERSON J LUND J OHLSEN A S A D KRISTENSEN H P O S multa neous deficiency of iron and vitamin B Dimorphic anemia Acta med scand na *163* 23 1959
49 TROWELL H C D morphic anaemia deficiency of iron a sociated with nutritional macrocytic anaemia Tr Roy Soc Trop Med & Hyg *3* 18 1913
50 THEORELL B Studies on the formation of cellular sub tances during blood production Acta med candina (suppl 200) *1* 9 1947
51 —— The relation of nucleic acid to the format on and d fferentiation of cellular protein Cold Spring Harbor Symposia on Quant Biol *12*:?47 1948
?2 WHITE J C LESLIE I AND DAVIDSON J N Nucleic ac d of bone marrow cells with pecial reference to pernicious anemia J Path & Bact *66* 291 19 3
3 MUELLER J F GLAZER H S AND VILTER R W Preliminary studies on the pur ne and pyrimidine ba es of human bone marrow as determined by paper chromatography I Variations in pern c ous anemia in response to therapy J Cl n In est *31* 6 1 1952
54 FRIEDKIN M AND WOOD H Conver on of uracil deox r bo ide to thym dine of deoxy ibosenucleic acid J Biol Chem 0 645 1956
4a SMITH L H JR AND BAKER F Pyr m line studies n pernicious anemia J Cl n In est *38* 1044 19 9
CASTLE W B Development of knowledge concerning the ga tric intrin ic factor and ts relat on to pernic us anemia New England J Med *49* 603 1953
6 GRAHAM R M AND RHEAULT M H Characteristic cellular changes in cells of nonhemopo etic origin in pernicious anem a J Lab & Clin Med *43* ?3 1954

19 Jones O 1 Origin of neutrophils in pernicious anemia (Cooke's macro polycytes) Arch Int Med *60* 1002 1937
20 Arneth J Qualitativ Blutlehre und Blutkrankheiten Leipzig J A Barth 1945
21 Wintrobe M M Diagnostic significance of changes in leucocytes Bull New York Acad Med *15* 223 1939
22 Storti L Diagnostic des Maladies du Sang translated into French by J Larghetta and A Drumas Paris G Doin 1953
23 Arneth J Über das qualitativ Verhalten der Blutplättchen bei der Perniciosa Folia haemat *57* 1 1937
24 Finch C A Coleman D H Motulsky A G Donohue D M and Reiff R H Erythrokinetics in pernicious anemia Blood *11* 807 1956
25 Cazal P Erythrocytes et Erythropathies Paris Masson 1957
26 Mallarmé J The study of the myelogram in pernicious anemia and the problem of the megaloblast Blood *3* 103 1948
27 —— and Debray J Les Anémies Mégaloblastiques Paris S E I E S 1949
28 Bessis M Cytology of the Blood and Blood Forming Organs translated into English by E Ponder New York Grune & Stratton 1956
29 Reisner E H Jr and Korson R Microspectrophotometric determination of desoxyribonucleic acid in megaloblasts of pernicious anemia Blood *6* 344 1951
30 Paulson M and Harvey J C Hematologic alterations after total gastrectomy evolutionary sequence over a decade J A M A *156* 1556 1954
31 Darby W J Bridgforth E B Le Brocquey J Clark S L Jr De Oliveira J D Kevany J McGanity W J and Perez C Vitamin B_{12} requirement of adult man Am J Med *25* 726 1958
32 Boen S T Molhuysen J A and Steenbergen J Nuclear changes in oral epithelial cells in subacute combined degeneration of the spinal cord due to vitamin B deficiency Lancet 2 294 1958
33 Holmes J M Cerebral manifestation of vitamin B deficiency Brit M J 1394 1956
34 Girdwood R H The occurrence of growth factors for Lactobacillus leichmanni streptococcus faecalis and leuconostoc citrovorum in the tissues of pernicious anemia patients and controls Biochem J 5 8 195
35 Swendseid M E Hvolboll E Schick G and Halsted J A The vitamin B_{12} content of human liver tissue and its nutritional significance a comparison study of various age groups Blood *12* 24 1957
36 Glass G B J Radioactive vitamin B in the liver III Hepatic storage and discharge of $CO^{60}B$ in pernicious anemia J Lab & Clin Med 5 87 1958
37 Schloesser L L Deshpande P and Schilling R F Biological turnover rate of cyanocobalamin (vitamin B) in human liver Arch Int Med *101* 306 1958
38 Glass G B J Deposition and storage of vitamin B in the normal and diseased liver Gastroenterology *36* 180 1959 Paper discussed by Halsted J A Popper H and Heinrich H C

5 BETHELL, F H MEYERS M C AND NELIGH R B Vitamin B_{12} in pernicious anemia and puerperal macrocytic anemia J Lab & Clin Med *33* 477 1948

6 DARBY W J JONES E CLARK S L MCGANITY W J DE OLIVEIRA J D PEREZ, C KEVANY J AND LE BROCQUY J The development of vitamin B_{12} deficiency by untreated patients with pernicious anemia Am J Clin Nutrition *6* 513 1958

77 JUKES T H AND WILLIAMS W L Occurrence of vitamin B_{12} in food *In* Sebrell W H and Harris R S The Vitamins New York Academic Press 1954 vol I

78 LEWIS U J REGISTER U D THOMPSON H T AND ELVEHJEM C A Distribution of vitamin B in natural materials Proc Soc Exper Biol & Med *7* 479 1949

79 KARLIN R La vitamine B_{12} dans le lait humain et bovin Compt rend Soc de biol *148* 371 1954

80 WOKES F BADENOCH J AND SINCLAIR H M Human dietary deficiency of itamin B_{12} Am J Clin Nutrition *3* 375 1955

81 GOUNELLE H AND RICHARD J Low serum itamin B_{12} concentrations in alcoholics improvement with liver therapy Am J Clin Nutrition *6* 492 1958

82 MOVITT E R Megaloblastic erythropoiesis in pat ents with cirrhosis of the liver Blood *5* 468 19 0

83 WATSON J LICHTMAN H C MESSITE J ELLISON R R CONRAD H AND GINSBERG V Clinical studies w th the citrovorum factor in megalobla tic anemia Am J Med *1* 17 19 4

84 JANDL J H AND LEAR A A The metabolism of folic acid in cirrhosis Ann Int Med *45* 1027 1956

8 WELLS R Nutrit onal vitamin B_{12} deficiency J Trop Med *61* 81 1958

86 MCINTYRE P KREVANS J R AND CONLEY C L Treatment of megaloblastic anem as J Chron Dis *6* 287 1957

87 POLLYCOVE M APT L AND COLPERT M J Pern cious anemia due to d etary deficiency of vitam n B_{12} New England J Med *255* 164 1956

88 ANDRUS E C AND WINTROBE M M Hyperthyroidism and pernicious anemia Bull Johns Hopkins Hosp *59* 291 19 6

89 BOENHEIM F SCHLIMER D AND MCGAVACK T H The combination of hyperthyro dism and pernicious anemia Ann Int Med 869 1945

90 ZIFFER H GUT IAN A PASHER I SOBOTKA H AND BAKER H Vit B in thyrotoxicos s and myxedema Proc Soc Exper Biol & Med *96* 229 19

91 GERSHOFF S N VITALE J J ANTONOWICZ I NAKA IURA M AND HELLER STEIN E E Studies of interrelationsh ips of thyroxine magnesium and vitamin B_{12} J Biol Chem *31* 849 19 8

92 FORSSELL J AND HALONEN I I Thyroid function and pernicious anemia Acta med scandinav *16* 61 19 8

93 ROSS G I M MOLLIN D L COX E V AND UNGLEY C C Hematologic

57 MASSEY B W AND RUBIN C E The stomach in pernicious anemia Am J M Sci 22, 481 1951
58 BOEN S T Changes in epithelial cells in vitamin B deficiency In Heinrich H C Vitamin B_{12} und Intrinsic Factor Stuttgart Ferdinand Enke 1957
59 GARDNER F H Observations on the cytology of gastric epithelium in tropical sprue J Lab & Clin Med 47 29 1956
60 JACOBSON W The mode of action of folic acid antagonists and the function of the Leuconostoc Citrovorum factor In Wolstenholme G E W and Cameron M P Ciba Foundation Symposium on Chemistry and Biology of Pteridines Boston Little Brown & Co 1954
61 STOKSTAD E L R Some properties of a growth factor for lactobacillus casei J Biol Chem 149 573 1943
62 PFIFFNER J J BINKLEY S B BLOOM E S BROWN R A BIRD O D AND EMMETT A D Isolation of the anti anemia factor (vitamin B) in crystal line form from liver Science 97 404 1943
63 RICKES E L BRINK N G KONIUSZY F R WOOD T R AND FOLKERS K Crystalline vitamin B_{12} Science 107 396 1948
64 SMITH E L Purification of anti pernicious anemia factors from liver Nature 161 638 1948
65 ARNSTEIN H R V The function of vitamin B_{12} and folic acid in the metabolism of one carbon units In Heinrich H C Vitamin B_{12} und Intrinsic Factor Stuttgart Ferdinand Enke 1957
66 BROQUIST H P Water soluble vitamins part I (folic acid B_{12} group choline) Ann Rev Biochem 27 28 19 8
67 ARNSTEIN H R V The function of vitamin B in animal metabolism Biochem Soc Symposia 13 92 1955
68 SHIVE W The functions of B vitamin in the biosynthesis of purines and pyrimidines Vitamins & Hormones 9 1951
69 JUKES T H AND STOKSTAD E L R The role of vitamin B in metabolic processes Vitamins & Hormones 9 1 1951
70 FORD J E AND HUTNER S H Role of vitamin B in the metabolism of microorganisms Vitamins & Hormones 13 101 1955
70a RUNDLE R W Hematopoietic effects of folic acid metabolites in the megaloblastic anemias Am J Clin Nutrition 3 385 1959
71 WILL J J MUELLER J F BRODINE C KIELY C F FRIEDMAN B HAWKINS R N DUBRA J AND VILTER R W Folic acid and vitamin B in pernicious anemia J Lab & Clin Med 53 22 1959
72 SMITH E L Vitamin B Brit M Bull 12 2 19 6
73 THOMAS E D AND LOCHTE H L JR Studies on the biochemical defect of pernicious anemia I In vitro observations on oxygen consumption heme synthesis and deoxyribonucleic acid synthesis by pernicious anemia bone marrow J Clin Invest 3 166 19 8
74 HALL B E AND CAMPBELL D C Vitamin B therapy in pernicious anemia I Effect on hematopoietic system preliminary report II Effect on the general clinical and neurologic manifestations preliminary report Proc Staff Meet Mayo Clin 3 584 1948 3 591 1948

containing food (extrinsic) factor upon intravenous injection without contact with gastric (intrinsic) factor J Lab & Clin Med *34* 1502 1949
108 HERBERT V In vitro organ specificity of intrinsic factor action Fed Proc *17* 440 1958
109 —— AND SPAET T H Distribution of intrinsic factor action Am J Physiol *195* 194 1958
110 —— On the mechanism of action of intrinsic factor Presented to Montefiore Hosp Research Soc December 20 1957
111 —— Mechanism of intrinsic factor action in the isolated rat small intestine J Clin Invest *37* 901 1958
112 —— Mechanism of intrinsic factor action in everted sacs of rat small intestine J Clin Invest *38* 102 1959
113 TAYLOR K B MALLETT B J AND SPRAY G H Observations on the inhibitory effects of intrinsic factor preparations on vitamin B absorption Clin Sc *17* 647 1958
114 —— Personal communication
115 ABELS J Intrinsic Factor van Castle en Resorptie van Vitamine B Doctoral Thesis University of Groningen Groningen V R B March 11 1959
115a COOPER B A AND CASTLE W B Factors influencing the action of intrinsic factor in vivo Clinical Research 206 1959
116 LATNER A L AND MERRILLS R J Further observations related to the isolation of intrinsic factor mucoprotein *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957
117 WILLIAMS W L AND ELLENBOGEN L Purification and assay of intrinsic factor *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957
118 GLASS G B J BOYD L J RUBINSTEIN M A AND SVIGALS C S Relationship of glandular mucoprotein from human gastric juice to Castle's intrinsic antianemia factor Science *115* 101 1952
119 WIJMENGA H G Intrinsic factor and vitamin B_{12} binding substance Purification, properties and possible relationship *In* Heinrich H C Vitamin B_{12} und Intrinsic Factor Stuttgart Ferdinand Enke 1957
120 FOX H J AND CASTLE W B Observations on the etiologic relationship of achylia gastrica to pernicious anemia IX Difference in site of secretion of intrinsic factor in the hog and in the human stomach Am J M Sc *203* 18 1942
121 LANDBOE-CHRISTENSEN E AND PLUM C M Experimental study on the localization of Castle's intrinsic factor in the human stomach Anti anemic effect of powdered human fundus and pylorus Am J M Sc *215* 17 1948 *ibid* 41 1950 Acta med scandinav *144* 167 1953 *ibid* *150* 369 1954
122 KEUNING F J ARENDS A MANDEMA E AND NIEWEG H O Observations on the site of production of Castle's intrinsic factor in the rat J Lab & Clin Med *53* 127 1959
123 McLEAN D L BAKER S J AND DONIACH I Addisonian pernicious anemia with gastric atrophy in a young man Brit J Haemat *1* 278 1955
124 FENWICK S On atrophy of the stomach Lancet 2 78 1870

responses and concentration of vitamin B in the *serum and urine* following oral administration of vitamin B without intrinsic factor Blood 9 4,3 19о4

94 UNGLAUB W G ROSENTHAL H L AND *GOLDSMITH* G A Studies of vitamin B in serum and urine following oral and parenteral administration J Lab & Clin Med *43* 143 19 4

95 DOSCHERHOLMEN A AND *HAGEN* P S A dual mechanism of vitamin B plasma absorption J Clin Invest *36* 1551 1957

96 ESTREN S BRODY E A AND WASSERMAN L R The metabolism of vitamin B in pernicious and other megaloblastic anemias Advances Int Med 9 11 1958

97 BAKER S J AND MOLLIN D L The relationship between intrinsic factor and the intestinal absorption of vitamin B_1 Brit J Haemat *1* 46 19

98 ADDISON T On the constitutional and local effects of disease of the suprarenal capsules London S Highley 1855 See also ADDISON T Anemia—disease of the suprarenal capsules Lond Med Gaz *43* 517 1849

99 BIERMER A Ober progressive perniciose Anamie Korresp schweiz Ärzte 2 15 1872

100 VON *BONSDORFF* B Pathogenesis of vitamin B deficiency with special reference to tapeworm pernicious anemia *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 19

101 MINOT C R AND MURPHY W P Treatment of pernicious anemia by a special diet J A M A *87* 4 0 1926

102 CASTLE W B Observations on the etiologic relationship of achylia gastrica to pernicious anemia I The effect of the administration to patients with pernicious anemia of the contents of the normal human stomach recovered after the ingestion of beef muscle Am J M Sci *178* 748 1929

103 —— AND TOWNSEND W C *Ibid* II The effect of the administration to patients with pernicious anemia of beef muscle after incubation with normal human gastric juice Am J M Sci *178* 764 1929

104 —— —— AND HEATH C W *Ibid* III The nature of the reaction between normal human gastric juice and beef muscle leading to clinical improvement and increased blood formation similar to the effect of liver feeding Am J M Sci *180* 30 1930

105 —— HEATH C W AND STRAUSS M B *Ibid* IV A biologic assay of the gastric secretion of patients with pernicious anemia having free hydrochloric acid and that of patients without anemia or with hypochromic anemia having no free hydrochloric acid and of the role of intestinal impermeability to hematopoietic substances in pernicious anemia Am J M Sci *18* 741 1931

106 BERK L CASTLE W B WELCH A D HEINLE R W ANKER R AND EPSTEIN M *Ibid* Activity of vitamin B as food (extrinsic) factor New England J Med 39 911 1948

107 GARDNER F H HARRIS J W SCHILLING R F AND CASTLE W B *Ibid* VI Hematopoietic activity in pernicious anemia of a beef muscle extraction

147 WILLIAMS A W COGHILL N F AND EDWARDS F The gastric mucosa in pernicious anemia biopsy studies Brit J Haemat 4 4 7 1958
148 FABER K The etiology and pathogenesis of achylia gastrica In Lectures in Internal Medicine New York Paul B Hoeber 1927
149 JOSKE R A FINCKH E S AND WOOD I J Gastric biopsy a study of 1 000 consecutive successful gastric biopsies Quart J Med 4 269 1955
150 ALSTED G Pernicious anemia after nitric acid corrosion of the stomach Lancet 1 6 1937
151 BEYERS M R DIEFENBACH W C L MARK H AND MEYER L M Interrelationship of folic acid and vitamin B_{12} in macrocytic anemia associated with linitis plastica Acta med scandinav 142 351 1952
152 GOLDHAMER S M Macrocytic anemia in carcinoma of the stomach apparently due to lack of intrinsic factor Am J M Sc 195 17 1938
153 KAPLAN H S AND RIGLER L G Pernicious anemia and carcinoma of the stomach autopsy studies concerning their relationship Am J M Sc 209 339 1945
154 MOSBECH J AND VIDEBAEK A Mortality from and risk of gastric carcinoma among patients with pernicious anemia Brit M J 2 390 1950
155 ZAMCHEK N GRABLE E LEY A AND NORMAN L Occurrence of gastric cancer among patients with pernicious anemia at the Boston City Hospital New England J Med 252 1103 1955
156 SNAPPER, I GROEN J HUNTER D AND WITTS L J Achlorhydria anemia and subacute combined degeneration in pituitary and gonadal insufficienc Quart J Med 6 195 1937 Lancet 307 1942
157 LEITHOLD S L DAVID D AND BEST W R Hypothyroidism with anemia demonstrating abnormal vitamin B_{12} absorption Am J Med 4 535 1958
158 HERBERT V Unpublished data
159 BOENHEIM F Addison s disease and pernicious anemia Endokrinologie 28 140 1951
160 BERLIN R Addison s disease familial incidence and occurrence in association with pernicious anemia Acta med scandinav 144 1 1952
161 PAULSEN M CONLEY C L AND GLADSDEN F S Absence of intrinsic factor from intestinal juice of patients following total gastrectomy Am J M Sc 220 310 1950
162 MCLEAN L D Incidence of megaloblastic anemia after subtotal gastrectomy New England J Med 257 262 1957
163 LOEWENSTEIN F Absorption of cobalt labeled vitamin B after subtotal gastrectomy Blood 13 339 1958
164 BADENOCH J EVANS J R RICHARDS W C D AND WITTS L J Megaloblastic anemia following partial gastrectomy and gastroenterostomy Brit J Haemat 1 339 1955
165 BRODINE C FRIEDMAN B I SAENGER E L AND WILL J J The absorption of vitamin B_{12} labeled with radioactive cobalt following subtotal gastrectomy J Lab & Clin Med 53 20 1959
165a FORSHAW J W B Hyperproteinaemia after partial gastrectomy corrected by gluten free diet Brit Med J 1050 1958

125 MAGNUS H A A re assessment of the gastric lesion in pernicious anemia J Clin Path *11* 289 1958
126 GLASS G B J Gastric atrophy Its clinical significance and methods of detection Am J Digest Dis 2 409 1957
127 SIURALA M Gastric lesion in some megaloblastic anemias Acta med scandinav *154* 337 1956
128 DAVIDSON W M B AND MARKSON J L The gastric mucosa in iron deficiency anemia Lancet 2 639 1955
129 WITTS L J Simple achlorhydric anemia Guy Hosp Rep *80* 253 1930
130 STEVENSON T D LITTLE J A AND LANGLEY L Pernicious anemia in childhood New England J Med *55* 1219 1956
131 FINNEY J O Pernicious anemia unassociated with achlorhydria Ann Int Med *12* 1551 1939
132 ASKEY J M Addisonian pernicious anemia without achlorhydria does it exist? Gastroenterology 2 1 1944
133 JACOBS A Acid secretion by the stomach in pernicious anemia Brit J Haemat *4* 465 1958
134 MACLACHLAN W W G AND KLINE T M Occurrence of anemia in 4 generations Am J M Sci *172* 533 1926
135 CALLENDER S T AND DENBOROUGH M A A family study of pernicious anemia Brit J Haemat *3* 88 1957
136 McINTYRE P A HAHN R CONLEY C L AND GLASS B Genetic factors in predisposition to pernicious anemia Bull Johns Hopkins Hosp *104* 309 1959
137 ARBO J AND MOHR J Pernicious anemia in monovular twins Acta genet et stat med *8* 105 19 8
138 HARRIS JONES J N SWAN H T AND TUDHOPE G R Pernicious anemia without gastric atrophy and in the presence of free hydrochloric acid report of a case Blood *1* 401 19
139 BAKER S J AND MOLLIN D L The relationship between intrinsic factor and vitamin B absorption Rev hémat *10* 160 19
140 MOLLIN D L BOOTH C C AND BAKER S J The absorption of vitamin B in control subjects in Addisonian pernicious anemia and in the malabsorption syndrome Brit J Haemat 3 412 19
141 LEE S HERBERT V AND WASSERMAN L R Unpublished case report
142 GOLDHAMER S M The presence of the intrinsic factor of Castle in the gastric juice of patients with pernicious anemia Am J M Sc *191* 40 1936
143 EDITORIAL Heredity in gastric cancer and pernicious anemia Lancet 1 1 19 4
144 MOSBECH J Heredity in Pernicious Anemia Copenhagen Ejnar Munksgaard 1953
145 UNGLEY C C Some current views on the origin of pernicious anemia and the absorption of vitamin B Tr New York Acad Sc *14* 1 1
146 CONNOR H M Hereditary aspect of achlorhydria in pernicious anemia J A M A *94* 606 1930

181 McIntyre P A Sachs M V Krevans J R and Conley C L Pathogenesis and treatment of macrocytic anemia Information obtained with radioactive vitamin B Arch Int Med 98 541 1956

182 Booth C C and Mollin D L The site of absorption of vitamin B_{12} in man Lancet *1* 18 1959

183 Latner A L Intrinsic factor and vitamin B absorption *Brit M J* *2* 278 1958

184 Reynell P C Spray G H and Taylor K B The site of absorption of vitamin B in the rat Clin S *16* 663 19[illegible]

185 Herbert V Castro Z and Wasserman L R Localization of the effect of intrinsic factor in the rat small intestine in vitro Clinical Research [illegible] 33 1959

186 Baker S J Mackinnon N L and Vasudeva P Site of absorption of orally administered vitamin B in dogs Indian J M Res *46* 812 1958

187 Callender S T and Spray G H Preparation of haemopoietically active extracts from faeces Lancet *1* 1391 1951

188 Ross G I M and Mollin D L *Vitamin* B_{12} *in* tissues in pernicious anemia and other conditions *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 195[illegible]

189 Ellenbogen L and Williams W L Personal communication See Tables 9 and 10

190 Glass G B J Differentiation of macrocytic anemias and detection of pernicious anemia and sprue in remissions by accelerated measurement of hepatic uptake *of radioactive Co* [illegible] B_{12} *In Proceedings of the Sixth International* Congress of the International Society of Hematology New York Grune & Stratton 1958

191 Oxenhorn S Estren S Wasserman L R and Adlersberg D Malabsorption syndrome intestinal absorption of vitamin B Ann Int Med *4*[illegible] [illegible] 1958

192 Cooke W T The malabsorption syndrome in man Pathogenesis—current concepts National Vitamin Foundation Symposium March 3 1959 Am J Clin Nutrition (in press)

193 Adlersberg D The malabsorption syndrome in man Clinical and pathological aspects National Vitamin Foundation Symposium March 3 1959 Am J Clin Nutrition (in press)

194 Gardner F H The malabsorption syndrome in man Hematologic aspect National Vitamin Foundation Symposium March 3 1959 Am J Clin Nutrition (in press)

195 French A B *The malabsorption syndrome in man Management* National Vitamin Foundation Symposium March 3 1959 Am J Clin Nutrition (in press)

196 Panel and Open Discussion The malabsorption syndrome in man National Vitamin Foundation Symposium March 3 1959 Am J Clin Nutrition (in press)

197 Adlersberg D Wang C I and Bossak E T Disturbance in protein and lipid metabolism in malabsorption syndrome J Mount Sinai Hosp (New

165b Lundh C Intestinal ligation and absorption after gastrectomy Acta chirurg scandinav (Suppl 231) 1958

166 Brown L B Jr and Justus B W *In vitro* absorption of radioiron by everted pouches of rat intestine Am J Physiol *194* 319 1958

166a Moore C V and Dubach R Resorption conservation elimination and physiological iron losses *In* Keiderling W Ed Eisenstoffwechsel Stuttgart G Thieme 1959

167 Badenoch J Evans J R and Richards W C D The stomach in hypochromic anaemia Brit J Haemat *3* 175 1957

168 Ei J G Mettier S R Harter H A and McBride A Pernicious anemia due to the presence of intrinsic factor inhibitor diagnosed in childhood with a 25 year follow up Clinical Research *7* 90 1959

169 Glass G B J Boyd L J Stephanson L and Jones E L Metabolic interrelations between intrinsic factor and vit B III B absorption at varied intrinsic factor doses Proc Soc Exper Biol & Med 88 1 1955

170 Callender S T and Evans J R Observations on the relationship of intrinsic factor to the absorption of labelled vitamin B from the intestine Clin Sc *14* 387 1955

171 Taylor K B Mallett B J Witts L J and Taylor W H Observations on vitamin B absorption in the rat Brit J Haemat *4* 63 1958

172 Williams W L Chow B F Ellenbogen L and Okuda K Intrinsic factor preparations which augment and inhibit absorption of vitamin B in healthy individuals *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957

173 Goldsmith G A Nutritional anemias with especial reference to vitamin B Am J Med *25* 680 1958

173a Herbert V Castro Z and Wasserman L R Unpublished data

174 Citrin Y DeRosa C and Halsted J A Sites of absorption of vitamin B J Lab & Clin Med *50* 667 1957

175 Halsted J A Carroll M and Drenick E J Absorption of radioactive vitamin B after total gastrectomy New England J Med 251 161 1954

176 Johnson P C and Berger E S Enhanced urinary excretion of Co^{60} vitamin B produced by delayed release capsules Blood *13* 1 1958

177 Bird O D and Hoevet B The vitamin B binding power of proteins J Biol Chem *190* 181 1951

178 Bauriedel W R Picken J C Jr and Underkofler L A Reactions of cyanocobalamin and aquocobalamin with protein Proc Soc Exper Biol & Med *91* 377 1956

179 Grasbeck R Studies on the vitamin B binding principle and other biocolloids of human gastric juice Acta med scandinav Suppl 314 1956

180 Castle W B Heath C W Strauss M B and Heinle R W Observations on the etiologic relationship of achylia gastrica to pernicious anemia VI Site of interaction of food and gastric factors failure of in vitro incubation to produce thermostable hematopoietic principle Am J M Sc *194* 618 1937

215 FROST J W AND GOLDWEIN M I Ob ervations on vitamin B absorption in primary pernicious an mia during administration of adrenocortical steroids New England J Med *258* 1096 1958

216 KEELE K D The prognosis and treatment of sprue in India Brit M J *1* 986 1949

217 HAZARI O K AND WOODRUFF A W Prognosi of tropical sprue Brit M J *2* 344 19 8

218 COMBINED STAFF CLINIC Malab orption syndrome Am J Med *15* 790 1953

219 FRENCH J M AND HAWKINS C F The gluten free diet in idiopathic steatorrhea Med Clin North American *41* 1 85 1957

19a SCHWARTZ M K SLEISENGER M F FERT J H ROBERTS K F RANDALL H T AND ALMY T P The effect of a gluten free diet on fat nitrogen and mineral metaboli m in patients with prue Gastroenterology *3* 32 1957

220 THAYSEN T E H Non Tropical Sprue London Oxford University Press 1932

221 ADLERSBERG D Introduction to symposium on the malabsorption syndrome J Mount Sinai Ho p (New York) *24* 177 1957 Simultaneously published *in Adlersberg D The Mal bsorption Syndrome New York Grune &* Stratton 1957

222 RUBIN C E BRANDBORG L L PHELPS P C AND TAYLOR H C JR In testinal similarities between celiac disease and idiopathic sprue J Clin Invest *37* 927 19 8

223 —— —— AND —— Is the inte t nal pathology of cel ac disease reversible? A prelim nary report Clinical Research 7 190 1959

224 FRENCH J M HAWKINS C F AND SMITH N The effect of a wheat gluten free diet in adult idiopath c steatorrhoea Quart J Med 6 481 1957

225 KELLEY M L AND TERRY R Clinical and histolog cal observations in fatal non tropical prue Am J Med 5 460 19 8

226 THOMPSON M W Heredity maternal age birth order in etiology of celiac disease Am J Human Genet *3* 1 9 1951

227 DAVIDSON L S P AND FOUNTAIN J R Incidence of prue syndrome with some observations on natural hi tory Brit M J *1* 1157 19 0

228 ANDERSON D H AND DISANT AGNESE P A Idiopathic celiac disease I Mode of onset and diagn os s Pediatric *11* 207 19 3

229 COOKE W T Conferen e Anaem a and the alimentary tract Lancet 2 255 19 8

230 FLUM E AND WARBURG E Hem tolcgical change e pecially megalocyt c anemia in regional ileit s Acta med scandinav *10* 449 1939

231 CAMERON D C WATSON C M AND WITTS L J Cl nical assoc ation of macrocyt c anemia with intestinal st icture and anastomoses Blood *4* 93 1949

232 THOMPSON R B AND UNGLEY C C Megaloblastic anemia associated with anatomic lesions in the mall inte tine Blood *10* 771 19 5

233 HALSTED J A LEWIS I M AND GASSTER M Absorption of rad oactive

York) 24 206 1957 Simultaneously published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 1957

198 Paterson J C S The sprue syndrome Am J M Sc 31 9. 1956

199 Hunter F M and Prevatt A L Diagnostic methods in intestinal malabsorption Am J M Sc 236 81 1958

199a Volwiler W Gastrointestinal Malabsorptive Syndromes Chicago Year Book Publishers 1959

200 Himes H W and Adlersberg D Pathologic changes in the small bowel in idiopathic sprue biopsy and autopsy findings Gastroenterology 35 14 1958

201 Shiner M Small intestinal biopsies by the oral route Histopathologic changes in the malabsorption syndrome J Mount Sinai Hosp (New York) 24 273 1957 Simultaneously published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 1957

202 Smith R B W Sprinz H Crosby W H and Sullivan B H Peroral small bowel mucosal biopsy Am J Med 25 391 1958

203 Shiner M Conference Anaemia and the alimentary tract Lancet 2 5 19 8

204 Cooke W T Adult coeliac disease and other disorders associated with steatorrhea Brit M J 2 261 1958

205 Butterworth C E and Perez Santiago E Jejunal biopsies in sprue Ann Int Med 48 8 1958

206 Herbert V Studies on the role of intrinsic factor in vitamin B ab orption transport and storage Symposium on Vitamin Interrelationships Medical College of Virginia Richmond October 24 1958 Am J Clin Nutrition 7 433 1959

207 Frost J W Goldwein M I and Kaufman B Studies of B Co60 absorption in malabsorption syndrome results before and during specific therapy Ann Int Med 47 293 1957

208 Joske R A Shamma'a M H and Drummey G D Intestinal malabsorption following temporary occlusion of the superior mesenteric artery Am J Med 25 449 19 8

209 Grasbeck R Kantero I and Siurala M Influence of calcium ion on vitamin B_{12} absorption in steatorrhea and pernicious anemia Lancet 1 234 19 9

210 Ashford B K Sprue In Tice's Practice of Medicine Hagerstown Md W F Prior vol 4 p 173

211 Spies T D Milanes F Menendez A Koch M B and Minnich V Observations on the treatment of tropical sprue with folic acid J Lab & Clin Med 31 27 1946

212 Stefanini M Clinical features and pathogenesis of tropical sprue Medicine 27 379 1948

213 Glass G B J Intestinal absorption and hepatic uptake of vitamin B in diseases of the gastrointestinal tract Gastroenterology 30 37 19 6

214 Doig A Girdwood R H Duthie J J R and Knox J D E Response of megaloblastic anemia to prednisolone Lancet 966 19

250 BADENOCH J. The blind loop syndrome *In* Jones F A. Modern Trends in Gastroenterology New York Paul Hoeber 1958

251 SIURALA M AND KAIPAINEN W J Intestinal megaloblastic anemia treated with aureomycin and terramycin Acta med scandinav *11* 19 1953

252 BADENOCH J BEDFORD P D AND EVANS J R Massive diverticulosis of the small intestine with steatorrhoea and megaloblastic anemia Quart J Med *4* 321 1955

253 BURKHOLDER P R Microbiological studies on materials which potentiate oral vitamin B therapy in addisonian anemia Arch Biochem *39* 322 1952

254 SYDERHELM R LEHMANN W AND WICHELS I Experimentelle intestinale perniziose Anamie beim Hund Klin Wchnschr 1439 1924

255 CAMERON D G WATSON G M AND WITTS L J The experimental production of macrocytic anemia by operations on the intestinal tract Blood *4* 803 1949

256 WATSON G M AND WITTS L J Intestinal macrocytic anemia Brit M J *1* 13 1952

257 LICHTMAN H GINSBERG V AND WATSON J Therapeutic effect of aureomycin in pernicious anemia Proc Soc Exper Biol & Med *4* 884 1950

258 FOY H AND KONDI A The haematinic action of penicillin in megaloblastic anemia and its relationship to B_{12} metabolism and the intestinal flora Tr Roy Soc Trop Med & Hyg *48* 17 1954

259 GIRDWOOD R H The megaloblastic anemias Quart J Med 5 8 1956

260 KILLANDER A Oral treatment of pernicious anemia with vitamin B_{12} and purified intrinsic factor I The value of serial estimation of the vitamin B levels of serum Acta med scandinav *160* 339 1958

261 HERBERT V WASSERMAN M AND SPAET T H Unpublished case report

262 WAGLE S R MEHTA R AND JOHNSON B C Vitamin B_{12} and protein biosynthesis V The site of action of vitamin B_{12} and its inhibition by a B_{12} antagonist Biochim et biophys acta *8* 15 1958

263 MULGAONKAR A G AND SREENIVASAN A Alterations in rat serum proteins in folic acid and vitamin B_{12} deficiencies Proc Soc Exper Biol & Med *94* 44 1957

264 BAKER H FRANK O PASHER I HUTNER S HERBERT V AND SOBOTKA H Substituted vitamin B_{12} amide I A microbiologic study Proc Soc Exper Biol & Med *100* 825 1959

265 HERBERT V HOOGSTRATEN B BAKER H AND WASSERMAN L R Patients given prolonged treatment with substituted amides of vitamin B_{12} in an attempt to control neoplastic growth [illegible]

266 OKUDA K HELLEGERS A E AND CHOW B F Vitamin B serum level and pregnancy Am J Clin Nutrition *4* 440 1956

267 BAKER H ERDBERG R PASHER I AND SOBOTKA H Study of folic acid and vit B_{12} in blood and urine during normal pregnancy Proc Soc Exper Biol & Med *94* 513 1957

268 BOGER W P BAYNE G M WRIGHT L D AND BECK G D Differential

vitamin B in the syndrome of megaloblastic anemia associated with intestinal stricture or anastomo is Am J Med 0 42 1956

234 KOGAN E SCHALIRA A JANOWITZ H D AND ADLERSBERG D Malabsorption following extensive small intestinal resection including inadvertent gastro ileostomy J Mount Sinai Hosp (New York) 24 339 1957 Simultaneously published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 1957

235 HARRISON H E HARRISON H C TOMISETT R R AND BARR D P Potassium deficiency in a ca of lymphosarcoma with the sprue syndrome Am J Med ? 131 1947

236 BJERKELUND C J Symptomatic prue Acta med candinav 137 130 19 0

237 HENDRIX J P BLACK SCHAFFER B WITHERS R W AND HANDLER P Whipple s intestinal lipodystrophy Arch Int Med 85 91 1950

238 OXENHORN S ESTREN S AND ADLERSBERG D Intestinal uptake of vitamin B in the malab orption syndrome J Mount Sinai Ho p (New York) 24 239 1957 Simultaneou ly published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 1957

239 BRUGSCH H Hyperchromic anemia in chronic disorders of pancreas Arch f klin Med 173 199 1932

240 KEFFER C S HUANG K K AND YANG C S Anemia associated with chronic dysentery Arch Int Med 4 436 1931

241 OKUDA K Vitamin B metabolism and thyroid hormones Nisshin Igaku 45 331 19 8

242 HERBERT V Stu lie of intrinsic factor mechanism of action Vitamin B Sympo ium New York Medical College April 11 1958 (in press)

243 GRASBECK R AND NYBERG W Inhibition of radiovitamin B ab orption by ethylenediaminetetraacetate (EDTA) and its reversal by calcium ions Scandina J Clin & Lab Inve t 10 448 19 8

244 HENNEMAN I H BENEDICT I H FORBES A I AND DUDLEY H R Idiopathic hypercal uria New England J Med 259 80 19 8

245 REISNER F H JR GILBERT J I RC ENBLUM C AND MORGAN M C Application of the urinary tracer test (of Schilling) a an index f vitamin B_{12} absorption Am J Clin Nutrition 4 134 19 6

24 a RUBIN C F Fers nal c mmunication

246 BRANTE C AND LR BERG I The in vitr uptake of vitamin B by Diphyllob thrium latum and it bl kage by intrin ic factor Scan lina J Clin & Lab Invest 9 313 19

247 NYBERG W The ab orpti n of ra lioacti e vitamin B in per n infe t l with the fish tapew rm Diphyllobothrium latum In H n el H C Vitamin B und Intrin i F ctor Stuttgart Ferdinan l Enke 19

248 BRANTE G AND ERNBERG T The mechani m of pernic u tap w rm anemia studied with C labele l vit min B Acta me l candin v 160 H 19 8

249 VON BONSDORFF B Perniciou a emia au e l by Diphyllob th i in the light of recent investi ations Bloo l 3 H 1948 Acta m l cand 144 903 1953

284 MILLER A The in vitro binding of cobalt60 labeled vitamin B_{12} by normal and leukemic sera J Clin Invest 37 556 1958

284a MILLER A AND SULLIVAN J F Some physicochemical properties of the vitamin B binding substances of normal and chronic myelogenous leukemic sera J Lab & Clin Med 53 607 1959

285 HORRIGAN D L AND HEINLE R W Refractory macrocytic anemia with defect in vitamin B_{12} binding and with response to normal plasma J Lab & Clin Med 40 811 1959

286 BETHELL F H Pteroylglutamic acid XI (B) Requirements of man In Sebrell W H and Harris R S The Vitamins New York Academic Press 1954 vol 3

287 WITTS L J Recent work on B vitamins in the blood and gastrointestinal tract especially in relation to human diseases Brit M Bull 1 14 1956

288 CHANARIN J Blood folic acid Brit M J 1 1179 1958

289 USDIN E PHILLIPS P N AND TOENNIES G Multiplicity of the folic acid active factors of blood J Biol Chem 221 865 1956

290 DENKO C W GRUNDY W E WHEELER N C HENDERSON C R FRIEDEMANN T E AND YOUMANS J B The excretion of B complex vitamins by normal adults on a restricted intake Arch Biochem 11 109 1946

291 BLANCK F C Handbook of Food and Agriculture New York Reinhold 1955

292 TOEPFER E W ZOOK E G ORR M L AND RICHARDSON L R Agriculture Handbook 29 United States Department of Agriculture 1951

293 SCHWEIGERT B S POLLARD A E AND ELVEHJEM C A The folic acid content of meats and the retention of this vitamin during cooking Arch Biochem 10 107 1946

294 IVES M POLLARD A E ELVEHJEM C A AND STRONG F M The nutritive value of canned foods XVII Pyridoxine biotin and folic acid J Nutrition 31 347 1946

295 STOKSTAD E L R Pteroylglutamic acid VIII Occurrence in foods In Sebrell W H Jr and Harris R S The Vitamins New York Academic Press 1954 vol 3

296 COLLINS R A HARPER A E SCHREIBER M AND ELVEHJEM C A The folic acid content and vitamin B_{12} content of the milk of various species J Nutrition 43 313 1951

297 ZUELZER W W Folic acid therapy in the anemias of infancy and childhood J A M A 131 7 1946

298 —— AND OGDEN F N Megaloblastic anemia in infancy a common syndrome responding specifically to folic acid therapy A M A Am J Dis Child 71 211 1946

299 LUHBY A L AND WHEELER W E Megaloblastic anemia of infancy II Failure of response to vitamin B_{12} and the metabolic role of folic acid and vitamin C Ohio State University Health Center J 3 1 1949

299a —— Megaloblastic anemia in infancy III Clinical considerations and analysis J Pediat 54 617 1959

serum vitamin B concentrations in mothers and infants New England J Med 256 1085 1957
269 PRYSTOWSKY H HELLEGERS A E RANKE E RANKE B AND CHOW B F Further observations on the metabolism of vitamin B_{12} in human pregnancy Am J Obst & Gynec 77 1 1959
269a BAKER H ZIFFER H PASHER I AND SOBOTKA H A comparison of maternal and foetal folic acid and vitamin B_{12} at parturition Brit M J 1 978 1958
270 HELLEGERS A OKUDA K NESBITT R E L JR SMITH D W AND CHOW B F Vitamin B_{12} absorption in pregnancy and in the newborn Am J Clin Nutrition 5 327 1957
271 LUHBY A L COOPERMAN J M DONNENFELD A M HERRERO J M TELLER D N AND WENIG J B Observations on transfer of vitamin B from mother to fetus and newborn Proc Soc Pediatric Res 28th Annual Meeting p 140 (May 6–7) 1958 A M A J Dis Child 96 532 1958
272 HUNT J N AND MURRAY F A Gastric function in pregnancy J Obst & Gynaec Brit Emp 65 78 1958
273 GILES C AND SHUTTLEWORTH E M Megaloblastic anaemia of pregnancy and the puerperium Lancet 1341 1958
274 IZAK G RACHMILEWITZ M STEIN Y BERKOVICI B SADOVSKY A ARONOVITCH Y AND GROSSOWICZ N Vitamin B and iron deficiencies in anemia of pregnancy and puerperium Arch Int Med 99 346 1957
275 HEINRICH H C Die biochemischen Grundlagen der Diagnostik und Therapie der Vitamin B Mangelzustände (B_{12} Hypo und Avitaminosen) des Menschen und der Haustiere Untersuchungen zum Vitamin B Stoffwechsel des Menchen während der Gravidität und Lactation Klin Wchnschr 3 205 1954
276 CALLENDER S T E A critical review of pernicious anemia of pregnancy Quart J Med 13 75 1944
277 DAS GUPTA C R CHATTERJEA J B AND BASU P Vitamin B in macrocytic anaemia in pregnancy Indian M Gaz 88 102 1953 Indian J M Res 42 411 1954
278 ISRAELS M C G AND DA CUNHA F A L Megaloblastic anaemia of pregnancy Lancet 2 214 1952
279 BADENOCH J CALLENDER S T EVANS J R TURNBULL A L AND WITTS L J Megaloblastic anaemia of pregnancy and the puerperium Brit M J 1 1245 1955
280 MOLLIN D L AND ROSS G I M Vitamin B deficiency in the megaloblastic anaemias Proc Roy Soc Med 47 428 1954
281 LOWENSTEIN L PICK C AND PHILPOTT M Megaloblastic anemia of pregnancy and the puerperium Am J Obst & Gynec 70 1309 1955
282 PITNEY W R BEARD M F AND VAN LOON E J Observations on the bound form of vitamin B_{12} in human serum J Biol Chem 207 143 1954
283 MENDELSOHN R S WATKIN D M HORBETT A P AND FAHEY J L Identification of the vitamin B_{12} binding protein in the serum of normal and of patients with chronic myelocytic leukemia Blood 13 740 1958

3 0 KILLANDER A Megaloblastic anemia associated with pregnancy or puerperium Report of three cases with normal serum vitamin B_{12} levels and subsequent response to treatment with vitamin B_{12} Acta haemat *19* J 1958

321 SCOTT J M Therapy in the megaloblastic anemias of pregnancy Obst & Gynec Surv *10* 370 1955

° MOORE C V VILTER R MINNICH V AND SPIES T D Nutritional macrocytic anemia in patients with pellagra or deficiency of the vitamin B complex J Lab & Clin Med *29* 12°6 1944

3°3 FOY H AND KONDI A Treatment of megaloblastic anaemias Relation of penicillin to vitamin B_{12} Lancet *2* 360 1953

°4 ADAMS E B Treatment of megaloblastic anaemia of pregnancy and the puerperium with vitamin B Brit M J *2* 398 1956

325 VILTER C F SPIES T D AND KOCH M B Further studies on folic acid in the treatment of macrocytic anemia South M J *38* 81 1945

3°6 GOODALL J W O GOODALL H I AND BANNERJEE D Folic acid in nutritional anaemia Lancet *1* °0 1948

3_7 RAMALINGASWAMI V AND MENON P S Folic acid in nutritional macrocytic anaemia Indian J M Res *3* 4/1 1949

3°8 SPIES T D DREIZEN S PARKER G S AND SILBERMAN D J Detection and treatment of nutritive failure in children Recent observations J A M A *148* 1376 1952

29 TASKER P W G Correlation of serum vitamin B_{12} levels and urinary folic acid in nutritional megaloblastic anaemia Lancet _ 0l 195

3 0 DAS GUPTA C R CHATTERJEA J B GHOSH S K AND BANNERJEE D K Vitamin B concentration of serum in nutritional megaloblastic anaemia Bull Calcutta Sch Trop Med *3* 101 1956

330a RUBIN C L BRANDBORG L L PHELPS P C AND TAYLOR H C JR Studies of celiac disease I The apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue Gastroenterology To be published

331 SPIES T D SUAREZ R M SUAREZ R M JR AND MORALES HERNANDEZ F The therapeutic effect of folic acid in tropical sprue Science *104* 75 1946

332 DARBY W J JONES E AND JOHNSON H C The use of synthetic L casei factor in the treatment of sprue Science *103* 108 1946

333 SPIES T D LOPEZ G G MENENDEZ A MINNICH V AND KOCH M The effect of folic acid on sprue South M J *39* 0 194C

334 MANSON BAHR P E C AND CLARKE O Folic acid in tropical sprue Lancet 903 1946

33 MORRISON R J G AND ST JOHNSTON C R Treatment of tropical sprue with folic acid Lancet *1* 636 1947

336 GIRDWOOD R H A folic acid excretion test in the investigation of intestinal malabsorption Lancet 65 53 1953

337 ANDERSON D H AND DI SANT AGNESE P A The celiac syndrome *In* McQuarrie I and Kelley V C Ed Brenneman s Practice of Pediatrics Hagerstown Md W F Prior 19 3 vol 1 chap 29

300 GASSER C Folsäure bei perniciosiformer Ziegenmilch Anämie Helvet paediat acta *3* 301 1948
301 BRAESTRUP P W C vitaminstudier hos spaedborn Dissert Copenhagen Haase & Son 1939
302 MONTO R W KAVANAUGH D AND REBUCK J W Severe nutritional macrocytic anemia in emotionally disturbed patients Am J Clin Nutrition *6* 105 1958
303 WALT F HOLMAN S AND HENDRICKSE R G Megaloblastic anaemia of infancy in kwashiorkor and other disease Brit M J *1* 1199 1956
304 CHANARIN I MOLLIN D L AND ANDERSON B B Folic acid deficiency and the megaloblastic anaemias Proc Roy Soc Med *51* 757 1958
305 LIEN KENG K AND ODANG O Megaloblastic anemia in infancy and childhood in Djakarta A M A Am J Dis Child *97* 709 1959
306 STURGEON P AND CARPENTER G Megaloblastic anemia of infancy Response to vitamin B Blood *5* 458 1950
307 GELIN G AND IBORRA M Les Anemies Megaloblastiques de l'Enfance et les Problemes de la Megaloblastose Paris Masson 1955
307a GERBASI M L anemie megaloblastique du nourrisson Rev port pediat e puer *21* 1 1958
308 BETHELL F H Pteroylglutamic acid IX (C) Effects of deficiency in man *In* Sebrell W H and Harris R S The Vitamins New York Academic Press 1954 vol 3
309 KOTHARI B V AND BHENDE Y M Nutritional megaloblastic anaemia Indian J M Res *37* 347 1949
310 UNGLEY C C The pathogenesis of megaloblastic anaemias and the value of vitamin B Brit J Nutrition *6* 299 19
311 GIRDWOOD R H Some aspects of disordered folic acid metabolism in man *In* Wolstenholme C E W and Cameron M P Ciba Foundation Symposium on Chemistry and Biology of Pteridines London J & A Churchill 1954
312 CHANARIN I ANDERSON B B AND MOLLIN D L The absorption of folic acid Brit J Haemat *4* 156 1958
313 COHEN I Post partum megaloblastic anemia South African M J *27* 697 1953
313a LOWENSTEIN L Vitamin B in pregnancy and the puerperium Am J Clin Nutrition (in press)
314 BERRY C G Anaemia of pregnancy in Africans of Lagos Brit M J 2 819 1955
315 CHAUDHURI S Vitamin B in megaloblastic anaemia of pregnancy and tropical nutritional macrocytic anaemia Brit M J 2 825 1951
316 PATEL J C AND KOCHER B R Vitamin B in macrocytic anemia of pregnancy and the puerperium Brit M J *1* 921 1950
317 VAN BUCHEM F S P AND NIEWIG H O Anaemia in pregnancy Lancet *1* 153 1959
318 VILTER R W Treatment of macrocytic anemia Arch Int Med *95* 48 1955
319 MOORE H C LILLIE E W AND GATENBY P B B The response of megaloblastic anemia of pregnancy to vitamin B_{12} Irish J M S *6* 106 1955

320 KILLANDER A Megaloblastic anemia associated with pregnancy or puerperium Report of three cases with normal serum vitamin B_{12} levels and subsequent response to treatment with vitamin B_{12} Acta haemat *19 J* 1958

321 SCOTT J M Therapy in the megaloblastic anemias of pregnancy Obst & Gynec Surv *10* 3,0 1955

322 MOORE C V VILTER R MINNICH V AND SPIES T D Nutritional macrocytic anemia in patients with pellagra o deficiency of the vitam n B complex J Lab & Clin Med *9* 12_6 1944

323 FOX H AND KONDI A Tr atment of megaloblastic anaem a Relation of penicillin to vitamin B_{12} Lancet ? 360 1953

324 ADAMS E B Treatment of megaloblastic anaemia of pregnancy and the puerperium with vitamin B_{12} Brit M J 3J8 19 6

325 VILTER C F SPIES T D AND KOCH M B Further studies on folic acid in the treatment of macrocytic anem a South M J *38 81 1945*

326 GOODALL J W O GOODALL, H I AND BANNERJEE D Folic acid in nutritional anaemia Lancet *1* 20 1948

327 RAMALINGASWAMI V AND MENON P S Fol c acid in nutritional macrocytic anaemia Indian J M Res *3*, 4,1 1949

328 SPIES T D DREIZEN S PARKER G S A D SILBER IAN D J Detection and treatment of nutr ti e failure in children Recent observation J A M A *148* 13 6 1952

329 TASKER P W G Correlation of serum vitamin B_{12} le eL and urinary folic acid in nutritional megaloblast c anaemia Lancet 61 19 5

330 DAS GUPTA C R CHATTERJEA J B GHO H S K AND BANNERJEE D K Vitamin B_{12} concentration of serum in nutritional megaloblastic anaem a Bull Calcutta Sch Trop Med *3* 101 19 6

330a RUBIN C E BRANDBORG I L PHELPS P C AND TAYLOR H C JR Studies of celiac d ea e I The pparent identical and specific nature of the duodenal and proximal jejunal lesion n celi c di ease and idiopathic prue Gastroenterology To be publ shed

331 SPIES T D SUAREZ R M SUAREZ R M JR AND MORALES HERNANDEZ F The therapeutic effect of folic acid n tropical sprue Science *104 5 1946*

332 DARBY W J JONES E AND JOHNSON H C The use of synthetic L casei factor n the treatment of sprue Sc ence *103* 108 1946

333 SPIES T D LOPEZ G G MENENDEZ A MINNICH V AND KOCH M The effect of folic acid on pru South M J *39* 0 1946

334 MANSON BAHR P E C AND CLARKE O Folic acid in trop cal prue Lancet *2 903 1946*

335 MORRISON R J G AND ST JOHNSON C R T eatment of tropical prue with folic acid Lancet *1* 636 1947

336 *GIRDWOOD R H A folic acid excretion test in the in estigation of intestinal* malabsorption Lancet *65* 53 19 3

337 ANDERSON D H AND DI SANT AGNESE P A The celiac syndrome *In McQuarrie I and Kelley V C Eds Brenneman s Practice of Pediatrics* Hagerstown Md W F Prior 1953 ol I chap 29

338 Di Sant Agnese P A Idiopathic celiac disease II Course and prognosis Pediatrics *11* 224 1953
339 Thompson L Folic acid therapy in coeliac disease Brit M J *1* 997 1948
340 Israels M C G and Sharp J Idiopathic steatorrhea (non tropical sprue) with megaloblastic anemia Lancet *1* 752 1950
341 Favorite G O The treatment of macrocytic anemia with synthetic L casei factor (folic acid) a case report Pennsylvania M J *49* 855 1946
342 Zumoff B Possible relationship of folic acid to uric acid metabolism as exemplified by a case of non tropical sprue Am J M Sc *225* 674 1953
343 Cohen B S Meyer L M and Fadem R Sprue refractory to vitamin B satisfactory response to folic acid Ann Int Med *36* 1533 1952
344 Davidson L S P Pteroylglutamic acid (folic acid) Therapeutic indications and limitations Edinburgh M J *55* 400 1948
345 —— Girdwood R H and Innes E M Folic acid in the treatment of the sprue syndrome Lancet 2 511 1947
346 Fox H J A comparison of pteroylglutamic acid and liver extract maintenance therapy in sprue New England J Med *240* 801 1949
347 Weir J F and Comfort M W Folic acid therapy in non tropical sprue Results of treatment in seven cases J Lab & Clin Med *32* 1231 1947
348 Adlersberg D and Schein J Clinical and pathologic studies in sprue J.A M A *134* 1459 1947
349 Thiersch J B and Philips F S Effects of 4 amino pteroylglutamic acid in dogs with special reference to megaloblastosis Proc Soc Exper Biol & Med *71* 484 1949
350 Nichol C A and Welch A D On the mechanism of action of Aminopterin Proc Soc Exper Biol & Med *74* 403 1950
351 Broquist H P Stokstad E L R and Jukes T H Some biological and chemical properties of the citrovorum factor J Biol Chem *185* 399 1950
352 Badenoch J The use of labelled vitamin B and gastric biopsy in the investigation of anaemia Proc Roy Soc Med *47* 4 6 1952
353 Hawkins C F and Meynell M J Macrocytosis and macrocytic anemia caused by anticonvulsant drugs Quart J Med *27* 45 1958
354 Berlyne N Levine M and McGlashen A Megaloblastic anemia following anticonvulsants Brit M J *1* 1247 19
355 Chalmers J N M and Bohfimer K Megaloblastic anemia and anticonvulsant therapy Lancet *2* 920 1954
356 Hawkins C F and Meynell M J Megaloblastic anemia due to phenytoin sodium Lancet *2* 737 1954
357 Rhind E G and Varadi S Megaloblastic anemia due to phenytoin sodium Lancet *2* 921 1954
358 Fuld H Megaloblastic anaemia following anticonvulsants Brit M J *1* 1175 1955
359 Girdwood R H and Lenman J A R Megaloblastic anaemia occurring during primidone therapy Brit M J *1* 146 1956
360 Newman M J D and Sweeney D W Megaloblastic anemia following the use of primidone Blood *12* 183 1957

361 CALVERT R J HERWORTH E AND MACBEAN A L Megaloblastic anemia from methophenobarbital Blood 13 894 1958

362 HOBSON Q J G SELWYN J G AND MOLLIN D L Megaloblastic anaemia due to barbiturates Lancet 271 1019 1956

363 CHRISTENSON W N ULTMANN J E AND ROSEMAN D M Megaloblastic anemia during primidone (mysoline) therapy J.A M.A 163 940 1957

364 WEBSTER J M Megaloblastic anaemia due to phenytoin sodium Lancet 2 1017 1954

365 CHANARIN I ELMES P C AND MOLLIN D L Folic acid studies in megaloblastic anaemia due to primidone Brit M J 2 80 1958

366 FALCO E A GOODWIN L G HITCHINGS G H ROLLO I M AND RUSSELL P B 2 4 diaminopyrimidines a new series of antimalarials Brit J Pharmacol 6 185 1951

367 MYATT A V HERNANDEZ T AND COATNEY C R Studies in human malaria XXXIII The toxicity of pyrimethamine (daraprim) in man Am J Trop Med 2 788 1953

368 TAHIE J LAPORTE J MONNIER J RIBET A BERNADET AND DAVÈRE Anémie avec megaloblastose medullaire au cours d un traitement par la pyriméthamine (malocide) Bull et mem Soc méd hop Paris 74 909 19 8

369 HITCHINGS G H ELION G B AND SINGER S Derivatives of condensed pyrimidine systems as antimetabolites In Wolstenholme G E M and Cameron M P Ciba Foundation Symposium on Chemistry and Biology of Pteridines Boston Little Brown & Co 1954

370 —— Daraprim as an antagonist of folic and folinic acid Tr Roy Soc. Trop Med & Hyg 46 467 19 2

371 WOOLLEY D W Antimetabolites Science 1 9 615 19 9

372 SPRAY G H AND WITTS L J Results of three years experience with microbiologic assay of vitamin B_{12} in serum Brit M J 1 295 1958

373 NICHOL G A AND WELCH A D Synthesis of citrovorum factor from folic acid by liver slice augmentation by ascorbic acid Proc Soc Exper Biol & Med 74 5° 1950

374 BROQUIST H P STOKSTAD F L R AND JUKES T H Biochemical studies with the citrovorum facto J Lab & Clin Med 38 9 1951

375 MCMILLAN R B AND INGLIS J C Scurvy a survey of fifty three cases Brit M J 2 233 1944

376 BRONTE STEWART B The anaemia of adult scurvy Quart J Med ? 309 1953

377 BROWN A Megaloblastic anaemia associated with adult scurvy report of a case which responded to synthetic ascorbic acid alone Brit J Haemat 1 345 1955

378 —— The anaemia of scurvy Glasgow M J 3 95 1951

379 METTIER S R MINOT G R AND TOWNSEND W C Scurvy in adults especially the effect of food rich in vitamin C on blood formation J.A M A 95 1089 1930

338 Di Sant'Agnese P A Idiopathic celiac disease II Course and prognosis Pediatrics 11 224 1953
339 Thompson L Folic acid therapy in coeliac disease Brit M J 1 29 1948
340 Israels M C G and Sharp J Idiopathic steatorrhea (non tropical sprue) with megaloblastic anemia Lancet 1 2 1950
341 Favorite G O The treatment of macrocytic anemia with synthetic L casei factor (folic acid) a case report Pennsylvania M J 49 855 1946
342 Zumoff B Possible relationship of folic acid to uric acid metabolism as exemplified by a case of non tropical sprue Am J M Sc 256/4 1953
343 Cohen B S Meyer L M and Fadem R Sprue refractory to vitamin B satisfactory response to folic acid Ann Int Med 36 1533 1952
344 Davidson L S P Pteroylglutamic acid (folic acid) Therapeutic indications and limitations Edinburgh M J 55 400 1948
345 —— Girdwood R H and Innes E M Folic acid in the treatment of the sprue syndrome Lancet 511 194
346 Fox H J A comparison of pteroylglutamic acid and liver extract maintenance therapy in sprue New England J Med 40 801 1949
347 Weir J F and Comfort M W Folic acid therapy in non tropical sprue Results of treatment in seven cases J Lab & Clin Med 3 1231 194
348 Adlersberg D and Schein J Clinical and pathologic studies in sprue J A M A 134 1459 1947
349 Thiersch J B and Philips F S Effects of 4 amino-pteroylglutamic acid in dogs with special reference to megaloblastosis Proc Soc Exper Biol & Med 1 484 1949
350 Nichol, C A and Welch A D On the mechanism of action of Aminopterin Proc Soc Exper Biol & Med 74 403 1950
351 Broquist H P Stokstad E L R and Jukes T H Some biological and chemical properties of the citrovorum factor J Biol Chem 185 399 1950
352 Badenoch J The use of labelled vitamin B and gastric biopsy in the investigation of anaemia Proc Roy Soc Med 4 4 6 1952
353 Hawkins C F and Meynell M J Macrocytosis and macrocytic anemia caused by anticonvulsant drugs Quart J Med 7 45 1958
354 Berlyne N Levine M and McGlashen A Megaloblastic anemia following anticonvulsants Brit M J 1 1247 1955
355 Chalmers J N M and Boheimer K Megaloblastic anemia and anticonvulsant therapy Lancet 920 1954
356 Hawkins C F and Meynell M J Megaloblastic anemia due to phenytoin sodium Lancet 2 37 1954
357 Rhind E G and Varadi S Megaloblastic anemia due to phenytoin sodium Lancet 921 1954
358 Fuld H Megaloblastic anaemia following anticonvulsants Brit M J 1 14 5 1955
359 Girdwood R H and Lenman J A R Megaloblastic anaemia occurring during primidone therapy Brit M J 1 146 1956
360 Newman M J D and Sumner D W Megaloblastic anemia following the use of primidone Blood 12 183 1957

erythrocyte *In* BEAN W B ED Monographs in Medicine Series I Baltimore Williams and Wilkins 1952

401b HUGULEY C M JR, BAIN J A RIVERS S L AND SCOGGINS R B Refractory megaloblastic anemia associated with excretion of orotic acid Blood *14* 615 1959

402 WATSON C J Macrocytic anemia Illinois M J 8 19 1942

403 DAMESHEK W AND INGALL M Agranulocytosis (malignant neutropenia) report of 9 cases 2 with recovery Am J M Sc *181* 502 1931

404 CONLEY C L GREEN T W HARTMAN R C AND KREVANS J R Prolonged treatment of pernicious anemia with vitamin B_{12} Am J Med *13* 284 1952

405 TE ITKA T AND BRAUN B Mechanism of increased hemolysis in pernicious anemia and autohemolytic action of liver Diet Folia haemat *48* 3 1932

405a CHEVALLIER P BERNARD J BILSKI PASQUIER G AND CHRISTOL D La tuberculose aigue pseudoleucosique Semaine Hop Paris *30* 114 1954

406 KASJIRSKY I A Der Punkt on des Knochenmarkes und die Blutbildung bei der visceralen Kinderleishmaniose D et Folia haemat *51* 352 1934

407 ROHR K Knochensmark morphologie des Menschlichen Sternalpunktats Berlin Urban & Schwartzenberg 1937

408 KOSZEWSKI B J The occurrence of megaloblastic erythropoiesis in patients with hemochromatosis Blood *7* 118 1952

409 GILLMAN T Cell enzymes and iron metabolism in anemias and siderosis Nutrit Rev *16* 3 3 1958

410 GRUELUND S Megaloblastic hemolytic anemia Acta med scandinav (suppl 2 9) *138* 101 19 0

411 DEMIRO P AND LEONARDI C Hemopathic mediterranean syndrome Acta med scandinav *138* 362 19 0

412 GERIOLA F Hemolytic splenomegaly associated with perniciosiform anemia Inform Med Genova *3* 17 1949

413 JANDL J H AND GREENBERG M S Bone marrow failure due to relative nutritional deficiency in Cooley s hemolytic anemia New England J Med *60* 461 1959

413a JOHNSON U ROATH O S AND KIRKPATRICK C I F Nutritional megaloblastic anemia associated with sickle cell states Blood *14* 535 19 9

414 GRANVILLE N AND DAMESHEK W Hemochromatosis with megaloblastic anemia responding to folic acid New England J Med *58* 586 1958

415 MAIER C Megaloblastic vitamin B deficiency anemia with hemochromatosis Schweiz med Wchnschr *8* 1234 1957

416 CASTLE W B *Commentary In Beeson I B Muschenheim C Castle* W B Harrison T R Ingelfinger F and Bondy P K The Yearbook of Medicine 1958–19 9 Series Chicago The Year Book Publishers 1958

417 MOHLER D N AND LEAVELL B S Aplastic anemia an analysis of 50 cases Ann Int Med *49* 390 19 8

418 MUELLER J F AND WILL J J Interrelationship of folic acid vitamin B_{12} and ascorbic acid in patients with megaloblastic anemia Am J Clin Nutrition *3* 0 1955

380 Crandon J H Lund C C and Dill, D B Experimental human scurvy New England J Med 223 353 1940

381 Krebs H A Peters R A Coward K H Mapson L W Parsons L C Platt B S Spence J C and O'Brien J R P Vitamin C requirement of human adults Experimental study of vitamin C deprivation in man Lancet 1 853 1948

382 Aldrich R A and Nelson E N Megaloblastic anemia in infants J Lancet 67 399 1947

383 May C D Nelson E N Lowe C U and Salmon R J Pathogenesis of megaloblastic anemia in infancy interrelationship between pteroylglutamic acid and ascorbic acid A M A Am J Dis Child 80 191 1950

384 Davis L J and Brown A The Megaloblastic Anaemias Springfield Ill Charles C Thomas 1953

385 Ehrlich P and Lazarus A Histology of the Blood Cambridge University Press 1900

386 Vaughan J M Leuco erythroblastic anemia J Path & Bact 4 541 19 C

387 Wilson I E Bone marrow in anemia M J Australia 1 513 1942

388 Baez Villasenor J and Sanchez Medal L Case report a case of erythemic myelosis (Di Guglielmo) Blood 8 1137 1953

389 Dameshek W and Gunz F Leukemia New York Grune & Stratton 19 8

390 Baldini M Fudenberg H Fukutake K and Dameshek W The anemia of the Di Guglielmo syndrome Blood 14 334 1959

391 Israel A Liver resistant pernicious anemia with megaloblastosis of bone marrow terminating in acute leukemia Rev hemat 4 655 1949

392 Sinn C M and Dick F W Monocytic leukemia Am J Med 0 88 19 6

393 Israels M C G Hemoglobinization of erythroblasts J Path & Bact 5 361 1941

394 Lambin P and De Weerdt W Le probleme des megaloblastes Rev belge c méd 10 282 1938

395 Stone F H Megaloblastic erythropoiesis in a case of myeloid leukemia during treatment Glasgow M J 31 1-5 19 0

396 Dameshek W and Baldini M The Di Guglielmo syndrome Blood 13 192 1958

397 Wasserman L R Personal communication supported by slides of peripheral blood and bone marrow morphology

398 Israels M C G and Wilkinson J F Achrestic anaemia Quart J Med 9 163 1940

399 Davidson L S P Refractory megaloblastic anemia Blood 3 107 1948

400 *Dacie J V Smith M D White J C and Mollin D L Refractory* normoblastic anaemia a clinical and haematological study of seven cases Brit J Haemat 5 56 1959

401 Roelson E and Ohlsen A S Achrestic anemia completely refractory megaloblastic anemia A case followed for nearly four years Acta med scandinav 150 17 19 4

401a Vilter R W and Mueller J F The growth and maturation of the

433 NEILL D W AND WEAVER J A Amino acid and protein metabolism in pernicious anemia Brit J Haemat 4 447 1958
434 FRASER M J AND HOLDSWORTH E S Vitamin B and protein biosynthesis in chick liver Nature 1 519 1959
435 ARNSTEIN H R V AND SIMKIN J L Vitamin B and protein biosynthesis in rat liver Nature 1 5 3 19 9
436 NEILL D W AND WEAVER J A A study of some metabolic processes dependent on coenzyme A in pernicious anemia Acta haemat 1 23 1959
437 SMITHBURN K C AND ZERFAS L C The neural symptoms and signs in pernicious anemia Arch Neurol & Psychiat 25 1110 1931
438 HYLAND H H WATTS G O AND FARQUHARSON R F Course of subacute *combined degeneration of spinal cord Canad M A J 65 995 1951*
439 DAVIDSON S Clinical picture of pernicious anemia prior to introduction of liver therapy in 1926 and in Edinburgh subsequent to 1944 Brit M J *1* 41 1957
440 GREENFIELD J C AND CARMICHAEL E A The peripheral nerves in cases of subacute combined degeneration of the cord Brain 58 483 1935
441 VAN DER SCHEER W M AND KOEK H C Peripheral nerve lesions in cases of pernicious anemia Acta psychiat et neurol 13 61 1938
442 JEWESBURY E C C Subacute combined degeneration of the cord and *achlorhydric peripheral neuropathies without anaemia Lancet 307 1954*
443 CASTLE W B The anemias *In* Cecil R L and Loeb R F A Textbook of Medicine ed 10 Philadelphia W B Saunders 1959
444 UNGLEY C C Treatment with vitamin B in haematological disorders *In* Heinrich H C Vitamin B and Intrinsic Factor Stuttgart Ferdinand Enke 19 /
445 HEATON J M MCCORMICK A J A AND FREEMAN A G Tobacco amblyopia a clinical manifestation of vitamin B_{12} deficiency Lancet 2 286 19 8
446 HAMILTON H E ELLIS P I AND SHEETS R F Visual impairment due to optic neuropathy in pernicious anemia report of a case and review of the literature Blood 14 378 1959
446a HARRIS C E C Snares and delusions in the recognition of pernicious anemia Connecticut Med J 12 543 1958
447 VICTOR M AND LEAR A A Subacute combined degeneration of the spinal cord Current concepts of the disease process Value of serum vitamin B *determinations in clarifying some of the common clinical problems* Am J Med 0 696 19 6
448 WOLTMAN H W AND HECK F J Funicular degeneration of spinal cord without pernicious anemia neurologic aspects of sprue nontropical sprue and idiopathic steatorrhea Arch Int Med 60 792 1937
449 ARIAS I M APT L AND LELLYCOVE M Absorption of radioactive vitamin B_{12} in nonanemic patients with combined system disease New England J Med 53 1005 1955
4 0 ELLENBOGEN L WILLIAMS W L RABINER S F AND LICHTMAN H C An

418a ARROWSMITH W R BURRIS M B AND SEGALOFF A Production of megaloblastic marrow by administration of cortisone in aplastic anemia with subsequent response to vitamin B relationship not previously described J Lab & Clin Med 42 ,,8 1953
419 CUSTER R 1 An Atlas of the Blood and Bone Marrow Philadelphia Saunders 1949
419a WEISSBACH H TOOHEY J AND BARKER H A Isolation and properties of B, coenzymes containing benzimidazole or dimethylbenzimidazole Proc Nat Acad Sc 45 521 1959
420 RICHMOND J AND DAVIDSON S Subacute combined degeneration of the spinal cord in non addisonian megaloblastic anaemia Quart J Med 27 517 1958
421 SUAREZ R M Sprue In Cecil R L and Loeb R F A Textbook of Medicine ed 9 Philadelphia W B Saunders 1955
422 NIEWIC H O VAN BUCHEM F S P AND KROESE W F Vitamin B and pteroylglutamic acid in the treatment of megaloblastic anemias Acta med scandinav 142 45 1952
423 —— FABER J C DE VRIES J A AND KROESE W F S The relationship of vitamin B and folic acid in megaloblastic anemias J Lab & Clin Med 44 118 1954
424 BODIAN D Nucleic acid in nerve cell regeneration Symp Soc Exper Biol 1 163 194
425 ALEXANDER W F Pathomorphology of the nervous system in vitamin B deficiency In Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957
426 ZIRM K L Uber die spezifische Anreicherung von Kobaltchlorophyllin im Nervensystem ihre therapeutische Bedeutung und Beziehung zum Vitamin B In Heinrich H C Vitamin B_{12} und Intrinsic Factor Stuttgart Ferdinand Enke 1957
427 CASPERSSON T O Cell Growth and Cell Function New York W W Norton 19 0
428 STERN J R TAYLOR M W AND RUSSEL W C Relation of vitamin b to liver baophilia Proc Soc Exper Biol & Med 0 551 1949
429 BAUER H AND HEINRICH H C Pathophysiologische Voraussetzungen zur therapeutischen Anwendung des Vitamin B bei neurologischen Erkrankungen In Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957
430 PRUSOFF W H TEPLY L J AND KING C C The influence of pteroyl glutamic acid on nucleic acid synthesis in Lactobacillus casei J Biol Chem 176 1309 1948
431 HODSON A Z Compounds with folic acid activity Arch Biochem 1 339 1949
432 WAGLE S R MEHTA R AND JOHNSON B C Vitamin B_{12} and protein biosynthesis VI Relation of vitamin B to amino acid activation J Biol Chem 233 619 1958

464 —— Leberkrankung und [illegible] erniziose Ana n e Blut 4 261 19 8

465 STEVENSON T D AND BEARD M F Serum vitamin B_{12} content in liver disease New England J Med 260 206 1959

465a DE GRUCHY G C Clinical Haematology in Medical Practice Springfield Ill C C Thomas 1958

466 CAMERON D G PENSLEY E H AND WOOD I Latent steatorrhea Ann Int Med 3 553 1952

467 JUERGENS J L SCHOLZ D A AND WOLLAEGER E E Severe osteomalacia associated with occult steatorrhea due to non tropical prue Arch Int Med 98 774 1956

468 STURGIS C C AND ISAACS R Pernicious anemia In Downey H Handbook of Hematology New York Paul B Hoeber 1938 vol 3

468a MARSHALL R A AND JANDL J H Diagno t c u e of small doses of folic acid in megaloblastic anemias Clinical Research 7 206 1959

468b MCKELLAR M Investigation of megaloblastic anemias New Zealand J Med 58 18 19 9

469 HAM T H A Syllabus of Laboratory Examinations n Clinical Diagnosis Cambridge Harvard University Press 19 2

470 BLOOMFIELD A L AND POLLARD W S Ga tric Anacidity New York Mac m llan 1933

471 SEGAL H L MILLER L L AND MORTON J J Determ nat on of gastric acidity without intubation by u e of cation exchange indicator compounds Proc Soc Exper Biol & Med 74 218 19 0

472 RECHTSCHAFFEN J S VENET L AND WEINGARTEN M Gastric acidity deter mination procedures Comparison of a tubeless method with azure A resin compound and an intubation method with histamine J A M A 164 1467 1957

473 BOLT R J OS IUS T G AND I LLARD H M A clinical evaluation of tubeless gastric analysis Gastroenterology 3 34 1957

474 DENBOROUGH M A RETIEF F P AND WITTS L J Tubele s ga tric analysis Brit M J 1 1 13 19 8

475 HERBERT V Development of a pos ble in vitro as ay for intr nsic factor Proc S c Exper Biol & Med 9 6 8 19 8

476 —— CASTRO Z AND WASSERMAN L R Rapid in vitro a ay of intrinsic factor in human ga tric ju ce Clinical Research 7 9 19 9

477 GRASBECK R Studie on the vitamin B 1 ding principle and other biocolloids of human gastric juice Acta med scand n v suppl 314 19 6

478 BERTCHER R W MEYER L M AND MILLER I F Co 60 vitamin B_{12} bind ng capacity of normal human sal va I c Soc Exper B ol & Med 99 513 19 8

479 OKUDA K GRASBECK R AND CHOW B F Bile and vitamin B absorption J Lab & Cl n Med 51 17 19 8

480 GRASBECK R NYBERG W AND REIZENSTEIN P Biliary and fecal vit B_{12} excretion in man An isotope study Proc Soc Exper Biol & Med 97 780 19 8

improved urinary excretion test as an assay for intrinsic factor Proc Soc Exper Biol & Med 89 3?7 1955
451 SCHILLING R F Intrinsic factor studies II The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12} J Lab & Clin Med 42 860 1953
452 ROSS G I M Vitamin B assay in body fluids using Euglena gracilis J Clin Path 5 250 1952
453 STRAY G H An improved method for the rapid estimation of vitamin B in serum Clin Sc 14 661 1955
454 BOSSAK E T WANG C I AND ADLERSBERG D Clinical aspects of the malabsorption syndrome (idiopathic sprue) Observations in 94 patients J Mount Sinai Hosp (New York) 4 ?86 1957 Simultaneously published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 1957
455 BETHELL F H AND HARRINGTON B D Incidence and significance of disease of gall bladder and liver in pernicious anemia Am J Digest Dis & Nutrition 1 ?56 1934
456 BROWN M R The pathology of the gastro intestinal tract in pernicious anemia and subacute combined degeneration of the spinal cord New England J Med 210 473 1934
4?7 BOYLES L A AND LAYNE J A The gall bladder in patients with pernicious anemia a study of non visualization and rate of emptying Gastroenterology 4 121 1945
458 SINGER K KING J C AND ROBIN S The life span of the megalocyte and the hemolytic syndrome of pernicious anemia J Lab & Clin Med 3 1068 1948
459 HAMILTON H E DECOWIN E L SHEETS R F JANNEY C D AND ELLIS J A Studies with inagglutinable erythrocyte counts VI Accelerated destruction of normal adult erythrocytes in pernicious anemia contribution of hemolysis to the oligocythemia J Clin Invest 33 191 19 4
460 LONDON I M AND WEST R The formation of bile pigment in pernicious anemia J Biol Chem 184 3 ? 1950
460a YOLLYCOVE M Bone marrow hemolysis in anemia J Clin Invest 38 1031 1959
461 INGELFINGER F J Diseases of the gallbladder and bile ducts In Harrison T R Adams R D Bennett I L Jr Resnik W H Thorn G W and Wintrobe M M Principles of Internal Medicine New York McGraw Hill 1958
461a WATSON C J Cholelithiasis Cholecystitis In CECIL R L AND LOEB R F Eds A Textbook of Medicine tenth ed Philadelphia W B Saunders 1959
462 SCHILLING R F AND HARRIS J W Liver function in untreated addisonian pernicious anemia J Lab Clin Med 40 718 1959
46 a TALBOTT J H Gout and blood dyscrasias Medicine 38 1 3 1959
463 REIMANN F Leberkrankung und perniziose Anamie In Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 195

plasma of folic acid injected intravenously in normal subjects and patients with megaloblastic anaemia Brit J Haemat 4 435 1958

498 Cox, E V Meynell M J Cooke W T and Gaddie R The folic acid excretion test in the steatorrhea syndrome Gastroenterology 35 390 1958

499 Borek B A and Waelsch H The enzymatic degradation of histidine J Biol Chem 05 4 9 1953

500 Tabor H and Mehler A H Isolation of N formyl L glutamic acid as an intermediate in the enzymatic degradation of L histidin J Biol Chem 01 559 1954

01 Silverman M Gardiner, R C and Bakerman H A The nature of glutam c acid excreted in folic acid deficiency J Biol Chem 194 81 1952

02 Tabor H Silverman M Mehler A H Daft F S and Bauer H L histidine conversion to a urinary glutamic ac d derivative in folic deficient rats J Am Chem Soc 75 756 1953

503 Daft F S Certain aspects of the relations between nutrition and liver function In Brauer R Liver Function A Symposium on Approaches to the Quantitat e Description of L ver Function Publication #4 Washin to D C American Institute of Biological Sciences 1958

04 Broquist H P Ev dence for the excret on of formiminoglutamic acid following folic acid antagonist therapy in acute leukemia J Am Chem Soc 78 6205 1956

505 Luhby A L Observations on the excretion of formiminoglutamic acid in folic ac d deficiency in man Cl nical Research Proceedings 5 8 19 7

06 Tabor, H and Wyngarden L A method for the determination of formiminoglutam c ac d in urine J Clin Invest 37 824 1958

07 Hiatt H H Goldstein M and Tabor H Urinary excretion of formimino gluta n c acid by human subjects after antifolic aci l therapy J Clin Invest 3 829 1958

508 Luhby A L Cooperman J M Teller D N and Donnenfeld A M Excretion of formiminoglutam c acid in folic cid deficiency tates J Clin Invest 37 915 19 8

509 Silverman M Gardiner R C and Condit P T A method for the detection of N formiminoglutamic acid in urine J Nat Cancer Inst 0 1 19 8

10 Broquist H P and Luhby A L Detection and isolation of formiminoglutamic acid from urine in folic acid defic ency in humans Proc Soc Exper Biol & Med 100 349 19 9

511 Luhby A L Cooperman J M and Teller D N Urinary excretion of fo mim noglutamic acid Appl cation in diagnosis of clinical folic acid def ciency Am J Clin Nutrition 7 397 1959

12 Herbert V Baker H Frank O Pasher I Wasserman L R and Sobotka H Unpublished data

513 Silverman M and Pitney A J Dietary methionine and the excretion of form minoglutamic acid by the rat J Biol Chem 33 11 9 1958

481 MILLER O N Studies on an interaction among serum protein materials containing intrinsic factor and vitamin B Arch Biochem 78 1957
482 —— AND UNGLAUB W G A study of etiology of macrocytic anemia J Clin Invest 36 915 1957
483 HUTNER S H CURY A AND BAKER H Microbiological assays Analyt Chem 30 849 1958
484 COATES M E AND FORD J E Methods of measurement of vitamin B Biochem Soc Symp 13 36 1955
485 MOLLIN D L AND ROSS G I M The pathophysiology of vitamin B deficiency in the megaloblastic anaemias In Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957
485a LOWENSTEIN L Personal communication
486 SOBOTKA H BAKER H AND ZIFFER H Vitamin B_{12} distribution between plasma and cells Vitamin B_{12} Symposium New York Medical College April 11 1958 Am J Clin Nutrition (in press)
487 HERBERT V Discussion of reference 486 Am J Clin Nutrition (In press)
488 —— Unpublished data
489 —— WASSERMAN L R FRANK O PASHER I AND BAKER H (Intro by SOBOTKA H) Value of fasting serum folic acid levels Fed Proc 18 246 1959
489a BAKER H HERBERT V FRANK O PASHER I WASSERMAN L R AND SOBOTKA H A microbiologic method for detecting folic acid deficiency in man Clin Chem 5 275 1959
489b HERBERT V BAKER H FRANK O PASHER I SOBOTKA H AND WASSERMAN L R The measurement of folic acid activity in serum a diagnostic aid in the differentiation of the megaloblastic anemias Blood (In press)
490 DENKO C W Pteroylglutamic acid clearance in normal adults J Appl Physiol 3 559 1951
491 SPRAY C H AND WITTS L J The utilization of folic acid given by mouth Clin Sc 11 273 1952
492 CLARK S L JR Oral folic acid tolerance test in normal human subjects and patients with pernicious anemia Proc Soc Exper Biol & Med 8 1953
493 DENKO C W GRUNDY W E PORTER J W BERRYMAN G H FRIEDEMAN T E AND YOUMANS J B The excretion of B complex vitamins in the urine and feces of seven normal adults Arch Biochem 10 33 1946
494 REGISTER U D AND SARETT H P Urinary excretion of vitamin B_{12} folic acid and citrovorum factor in human subjects on various diets Proc Soc Exper Biol & Med 7 837 1951
495 SUAREZ R M WELCH A D HEINLE R W SUAREZ R M JR AND NELSON E N Effectiveness of conjugated forms of folic acid in the treatment of tropical sprue J Lab & Clin Med 31 1294 1946
496 SWENSEID M E BIRD O D BROWN R A AND BETHELL F H Metabolic function of pteroylglutamic acid and its hexaglutamyl conjugate J Lab & Clin Med 3 23 1947
497 CHANARIN I MOLLIN D L AND ANDERSON B B The clearance from the

pernicious anemia to pteroylglutamic acid and vitamin B J Lab & Clin Med 34 439 1949
9 Wolff R La position du foie dans le métabolisme normal et pathologique de la vitamine B In Heinrich H C Vitamin B_{12} und Intrinsic Factor Stuttgart Ferdinand Enke 1957
530 Darby W J Folic acid and citrovorum factor in human nutrition Nutrition Symp Series No 7 New York National Vitamin Foundation Inc 19 3
531 Unglaub W G and Goldsmith G A Folic acid and vitamin B in medical practice J A M A 161 6 3 1956
532 Ross J F Belding H and Paegel B L The development and progression of subacute combined degeneration of the spinal cord in patients with pernicious anemia treated with synthetic pteroylglutamic (folic) acid Blood 3 68 1948
533 Wright L D Skeggs H R and Huff J W The ability of thymidine to replace vitamin B as a growth factor for certain lact bacilli J Biol Chem 175 4 5 1948
534 Bok J Faber J G De Vries W F Kroese W F S and Niewig H O The effect of pteroylglutam c ac d on the serum vitamin B_{12} concentration in pernicious nemia i rel pse J Lab & Clin Med 51 667 19 8
535 A M A Council on Foods and Nutrition Vitamin preparations as dietary supplements and as therapeutic agents J A M A 169 41 1959
536 Moore C V Pernicious anemia In Conn H F Current Therapy 1959 Philadelphia W B Saunders 19 9
537 Harris J W Aggravation of clinical man festations of folic acid deficiency by small daily doses of v tam n B Am J Med 1 461 19 6
538 Cox E V Meynell M J Cooke W T and Gaddie R Folic acid and cyanocobala n n perniciou anaemia Clin Sc 17 693 19 8
539 Nelson R S and Doctor V M The v ta n n B_{12} content of hu nan liver as determined by bio assay of needle biopsy material Ann Int Med 49 1361 1958
540 Crossowicz N Hochman A Aronovitch J Izak G and Rachmilewitz M Mal gnant gro th in tl e l er and erum vitamin B_{12} le els Lancet 1 1116 1957
541 Conley C L Krevans J R Chow B F Burrows C and Lang C A Observat ons on the absorption utilization and excretion of vitamin B_{12} J Lab & Clin Med 38 84 19 1
542 Chesterman D C Cuthbertson W F J and Pegler H F V tamin B excretion tudies Biochem J 48 1i 19 1
543 Reisner E H Jr and Weiner L The treatment of pernicious anem a with ma si e parenteral do es of vita n n B_{12} Blood 8 81 1953
544 Sokoloff M S Sanneman E H Jr and Beard M F Urinary excretion of v tamin B Blood 7 43 19 9
545 Mollin D L and Ross G I M Vitam n B_{12} concentrations of serum and urine in the first 4 hour after intramuscular njection of the vitamin J Clin Path 6 54 1953

514 RABINOWITZ J AND TABOR H The urinary excretion of formic acid and formiminoglutamic acid in folic acid deficiency J Biol Chem 233 259 1958

514a HIATT H H RABINOWITZ J C TOCH R AND GOLDSTEIN M Effects of folic acid antagonist therapy on urinary excretion of formic acid by humans Proc Soc Exper Biol & Med 98 144 1958

515 HEINLE R W WELCH A D SCHARF V MEACHAM C C AND PRUSOFF W H Studies of excretion (and absorption) of Co 60 labeled vitamin B in pernicious anemia Tr A Am Physicians 65 214 1952

516 BOOTH C C AND MOLLIN D L Plasma tissue and urinary radioactivity after oral administration of 56 Co labelled B_{12} *In* Heinrich H C Vitamin B und Intrinsic Factor Stuttgart Ferdinand Enke 1957

517 GLASS G B J BOYD L J GELLIN G A AND STEPHANSON L Uptake of radioactive vitamin B by the liver in humans test of measurement of intestinal absorption of vitamin B and intrinsic factor activity Arch Biochem 51 2 1 1 4

518 ELLENBOGEN L AND WILLIAMS W L *Quantitative assay of intrinsic factor* by urinary excretion of radioactive vitamin B Blood 13 582 1958

519 REISNER E H JR ROSENBLUM C AND MORGAN M C Urinary excretion of orally administered Co 60 vitamin B in normal subjects and patients with pernicious anemia and sprue Clinical Research Proceedings 56 19 4

520 RATH C E McCURDY P R SCHREINER C E AND DUFFY B J The effect of renal disease on the urinary excretion of cobalt 60 vitamin B *In* Proceedings of the Sixth International Congress of the International Society of Hematology New York Grune & Stratton 1958

521 DUNN A L WALSH J R AND HOLTHAUS J M Radioactive cyanocobalamin (vitamin B) in renal disease Arch Int Med 101 9 1958

522 ROSENBLUM C AND CHTHARD L Personal communication

523 BUCHHOLZ C H Concentration of vitamin B from urine by adsorption on carbon J Lab & Clin Med 5 6 8 1958

524 HAWKINS C F Value of serum iron levels in assessing effect of haematinic in the microcytic anaemias Brit M J 1 383 1955

525 VILTER R W HORRIGAN D MUELLER J F JARROLD T VILTER C F HAWKINS V AND SEAMAN A Studies on the relationships of vitamin B folic acid thymine uracil and methyl group donors in persons with pernicious anemia and related megaloblastic anemia Blood 5 695 1950

526 CONLEY C L AND KREVANS J R Development of neurologic manifestations of pernicious anemia during multivitamin therapy New England J Med 45 29 1951

527 BERK L DENNY BROWN D FINLAND M AND CASTLE W B Effectiveness of vitamin B in combined system disease Rapid regression of neurologic manifestations and absence of allergic reactions in a patient sensitive to injectable liver extracts New England J Med 9 3 8 1948

528 SCHIEVE J F AND RUNDLES R W Response of lingual manifestations of

treatment of pernicious anemia with vitamin B_{12} without intrinsic factor New England J Med 253 509 1955
563 MILLER O N RANEY J L AND HUNTER F M Effect of intrinsic factor on uptake of radioactive vitamin B_{12} by slices of rat liver Fed Proc *16* 393 1957
564 —— AND HUNTER F M Stimulation of vit B_{12} uptake in tissue slices by intrinsic factor concentrate Proc Soc Exper Biol & Med *96* 39 1957
565 BASTRUP MADSEN P AND PAULSEN L Oral treatment of megaloblastic anaemia with small amounts of vitamin B_{12} and intrinsic factor Acta haemat *13* 193 1955
566 SCHWARTZ M LOUS P AND MEULENGRACHT E Reduced effect of heterologous intrinsic factor after prolonged oral treatment in pernicious anemia Lancet *1* 751 1957
567 KRISTENSEN O LUND J OHLSEN A S AND PEDERSON J Maintenance therapy in pernicious anemia controlled by determining vitamin B_{12} level in plasma Lancet *1* 1266 1957
568 LOWENSTEIN L BRUNTON L SHAPIRO L DE LEEUW N AND DUFRESNE M Maintenance therapy of pernicious anaemia with oral administration of intrinsic factor and vitamin B_{12} Canad M A J *77* 3 5 1957
569 BERLIN R BERLIN H BRANTE G AND SJOBERG S G Failures in long term treatment of pernicious anemia with B_{12} intrinsic factor preparations Acta med scandinav *161* 143 1958
570 SCHWARTZ M LOUS P AND MEULENGRACHT E Absorption of vitamin B_{12} in pernicious anemia Defective absorption induced by prolonged oral treatment Lancet 1 00 1958
571 STOKES J B AND FITNEY W R Pernicious anaemia treated orally with Bifacton Refractoriness to potent animal intrinsic factor Brit M J *1* 3 2 1958
LICHTMAN H C ELLENBOGEN L AND WILLIAMS W L Absorption of vitamin B_{12} by pernicious anemia patients before and after maintenance on an intrinsic factor concentrate Third Vitamin B_{12} Symposium New York Academy of Medicine (Jan 31 Feb 1) 1958 See also WILLIAMS W L Absorption of vitamin B_{12} via intrinsic factor concentrate Fed Proc *18* 551 1959
573 FOUTS P J HELMER O M AND CHERNISH S M Absorption of radioactive B_{12} in patients with pernicious anemia after long term oral and parenteral therapy The American College of Physicians Fortieth Annual Session Chicago (April 20–24) 1959
574 BERLIN R BERLIN H BRANTE G AND SJOBERG S G Refractoriness to intrinsic factor B_{12} preparations abolished by massive doses of intrinsic factor Acta med scandinav *16* 317 1958
575 SCHWARTZ M Intrinsic factor inhibiting substance in serum of orally treated patients with pernicious anemia Lancet *2* 61 1958
576 TAYLOR K B AND MORTON J A An antibody to Castle's intrinsic factor Lancet *1* 3 1958
577 HERBERT V Unpublished data

546 Mollin D L and Ross G I M Serum vitamin B_{12} concentration of patients with megaloblastic anaemia after treatment with vitamin B_{12} folic acid or folinic acid Brit M J 2 640 1955
547 Ungley W C Miller O N and Goldsmith G A Saturation studies with vitamin B in human subjects Am J Clin Nutrition 6 535 1958
548 Goodman L S and Gilman A The Pharmacological Basis of Therapeutics ed 2 New York Macmillan 1955
549 Goldblatt S On the intravenous administration of cyanocobalamin Am J Clin Nutrition 3 129 1955
550 Chow B F Personal communication to reference 482
551 Hale B F Krusen F H and Woltman H W Vitamin B_{12} and coordination exercises for combined degeneration of the spinal cord in pernicious anemia J A M A 141 257 1949
552 Crasbeck R Maintenance treatment in pernicious anaemia Lancet 1 206 1959
552a Rundles R W Prognosis in neurologic manifestations of pernicious anemia Blood 1 209 1946
552b Bethell F H and Sturgis C The relation of therapy in pernicious anemia to changes in the nervous system Blood 3 57 1948
553 O'Connor J S Davis R L Langworthy O R and Chow B F Metabolic studies of vitamin B_{12} and Depinar in multiple sclerosis (MS) and clinically healthy subjects Fed Proc 17 528 1958
553a Thompson R L and Hecht R A Studies on a long acting vitamin B_{12} preparation Am J Clin Nutrit 7 311 1959
554 Hemsted F H and Mills J Vitamin B in pernicious anemia Lancet 2 1302 1958
555 Nieburgs H E Cytologic technic for office and clinic Postgrad Med 23 309 1958
556 Tanaka K R and Clifford G O Spontaneous remission in pernicious anemia Report of a case New England J Med 258 77 1958
557 Glass G B J Biochemistry and physiology of Castle's intrinsic factor and its relationship to the metabolism of vitamin B Rev hemat 10 13 1955
558 Estren S and Wasserman L R Pernicious anemia I Remission by small oral doses of purified vitamin B Proc Soc Exper Biol & Med 91 499 1956
558a Schwartz S O Friedman I A and Cant H I Long term evaluation of vitamin B in treatment of pernicious anemia J A M A 157 229 1955
559 Chalmers J N M and Shinton N K Absorption of orally administered vitamin B_{12} in pernicious anemia Lancet 1298 1958
560 Brody E A Estren S and Wasserman L R Treatment of pernicious anemia by oral administration of vitamin B without added intrinsic factor New England J Med 260 361 1959
561 Conley C L and Krevans J R New developments in the diagnosis and treatment of pernicious anemia Ann Int Med 47 78 19
562 Reisner E H Jr Weiner L Schittone M T and Henck E A Oral

tions on the metabolism of f lic acid in man and on the effect of aminopterin C ncer 11 5 5 19;8

596 JUKES T H Recent studies with hemopoietic factors intrinsic factor and folic acid In Proceedings of the Sixth International Congress of the International Society of Hematology New York Grune & Stratton 19 8

597 NOREN B Allergic reactions in parenteral liver therapy and vitamin B Acta med scandinav 137 48 1950

598 OSTLING G NYBERG W AND GORDIN R Antianemic activity of alkali treated crude liver extract Acta med scandinav 145 40 19 3

599 OWREN P A A protein synthesis liver factor lacking in pernicious anemia and related macrocytic anemias Scandinav J Clin & Lab Invest 241 19 0

600 —— Is B the complete therapeutic answer in pernicious anemia? In Proceedings of the Third International Congress of the International Society of Hematology New York Grune & Stratton 19 1

601 GORDIN R Prothrombin in cryptogenic pernicious anemia and pernicious tapeworm anemia and its response to treatment Acta med scandinav 149 1 1954

602 BEZMAN A KINNEAR D G AND ZAMCHECK N D xylose and potassium iodide absorption and serum carotene in pernicious anemia J Lab & Clin Med 53 996 1959

603 SPAET T H AND KROPATKIN M Studies on prothrombin derivatives in vitamin K deficiency A M A Arch Int Med 102 558 19 8

604 WANG C I AND BOSSAK E T Hemorrhagic manifestations in idiopathic sprue a report of 9 cases and review of the literature J Mount Sinai Hosp (New York) 4 317 19 7 Simultaneously published in Adlersberg D The Malabsorption Syndrome New York Grune & Stratton 19 7

605 VILTER R W Ascorbic acid deficiency (scurvy) In Harrison T R Adams R D Bennett I L Jr Resnik W H Thorn G W and Wintrobe M M Principles of Internal Medicine New York McGraw Hill 1958

606 —— Ascorbic acid IX Effects of deficiency in human beings In Sebrell W H Jr and Harris R S The Vitamins New York Academic Press 19 4 vol 1

607 —— Ascorbic acid XI (B) Requirements of human beings In Sebrell W H Jr and Harris R S The Vitamins New York Academic Press 1954 vol 1

608 COX E V GADDIE R MATTHEWS D COOKE W T AND MEYNELL M J An inter relationship between ascorbic acid and cyanocobalamin Clin Sc 1 681 19 8

609 WALLERSTEIN R O HARRIS J W AND CABEZDA C J Ascorbic acid deficiency in pernicious anemia Am J Med 11 53 19 3

610 CROSBY W H Misuse of blood transfusion Blood 13 1198 1958

611 ADLERSBERG D COLCHER H AND WANG C Oral use of hydrocortisone (Compound F) in treatment of sprue Arch Int Med 92 615 1953

612 WOLLAEGER E E Sprue and allied malabsorption syndromes In Cecil R L AND LOEB R F Eds A Textbook of Medicine ed 10 Philadelphia W B Saunders 1959

578 Gyorgy P Rocantins L M Miller F R and Weiss A Hematopoiesis blood group substance and bifidus factor Tr A Am Physicians 67 117 1954

579 Latner A L Merrills R J and Raine L C D L Further observations on the isolation and chemical composition of Castle s intrinsic factor Biochem J 57 xix 1954

580 Stringer G F Uber fucosehaltige Mucine vorwiegend entodermalen Ursprungs mit Blutgruppen und anderen biologischen Eigenschaften Klin Wchnschr 33 317 1955

581 Van Baal J P W Wijmenga H G and Van der Hart M Blood group substance activity of intrinsic factor preparations Biochim et biophys acta 17 600 1955

581a Taylor K B and Morton J A An antibody to Castle s intrinsic factor J Path & Bact 77 117 1959

581b Lowenstein L Cooper B A Brunton L and Gartha S Antibodies in human and rabbit sera to hog intrinsic factor preparations J Clin Invest 38 1023 1959

582 Heathcote J G and Mooney F S The oral treatment of pernicious anemia Lancet 1 982 19 8

583 Nyberg W and Reizenstein P Intestinal absorption of radiovitamin B bound in pig liver Lancet 2 832 1958

584 Castle W B Oral treatment of pernicious anemia Lancet 2 270 1958

585 Latner A L Absorption of bound radioactive vitamin B Lancet 2 961 1958

586 Grasbeck R Reizenberg L Simons K and Nyberg W Absorption of bound radioactive vitamin B Lancet 2 961 1958

587 Chow B F Meier P and Free S M Absorption of vitamin B enhanced by d sorbitol Am J Clin Nutrition 6 30 19 8

588 Herbert V Bilerfass M Wasserman L R Estren S and Brody F Effect of d sorbitol on absorption of vitamin B by human subjects able to produce intrinsic factor Am J Clin Nutrition 7 395 19 9

589 Ellenbogen L Herbert V and Williams W L Effect of sorbitol on absorption of vitamin B by pernicious anemia patients Proc Soc Exper Biol & Med 99 9 7 19 8

590 Chalmers J N M and Shinton N K Effect of d sorbitol on the absorption of orally administered vitamin B Nature 183 118 19 9

591 Herbert V Sorbitol and vitamin B absorption Am J Clin Nutrition 6 547 1958

592 The Pharmacopeia of the United States of America Fifteenth Revision Official from December 1 19

593 Jukes T H B Vitamins for Blood Formation Springfield Ill Charles C Thomas 1952

594 Schoenbach E B Greenspan F M and Colsky J Reversal of Aminopterin and Amethopterin toxicity by citrovorum factor J A M A 144 1558 19 0

595 Condit P T and Creb D Studies on the folic acid vitamins I Observa

Index